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Age variation of the third upper molar in *Eothenomys smithii*

Yukibumi KANEKO

Biological Laboratory, Faculty of Education, Kagawa University, Takamatsu 760, Japan
Fax. 0878-36-1652

Abstract. A study was made of age variation in the size and enamel patterns of the third upper molar of 99 *Eothenomys smithii* specimens from Japan. No significant age variation was found in either the frequency of the patterns, or the width of the dentine confluent space between the second and the third triangles. Deep lingual reentrant folds, on the posterior loop, appear in specimens where the condylobasal length (CBL) is of 22-24 mm, then the pattern with a shallow reentrant fold increases in frequency in larger CBL classes. The depth of the inner fold showed the same tendency as the changes in the patterns. A significant association, however, between five enamel patterns and age classes, depends on classification according to CBL or body weight. This proved insignificant in five CBL classes, but significant in three CBL or body weight classes. A gradual transition in the age variation of the posterior loop patterns was found among *Eothenomys* species which have rootless molars throughout life. The simple enamel pattern form significantly increased in frequency with advancing age in *E. andersoni* and *E. shanseius*, resembling *Clethrionomys glareolus* and *C. rufocanus* ; on the other hand, in *E. regulus*, *E. inez*, *E. eva*, *E. chinensis*, *E. wardi*, *E. custos* and *E. proditor* no age variation was found on the posterior loop, thus resembling *Microtus pennsylvanicus*. *E. smithii* shows a little age variation in the enamel patterns, the variation of which is of an intermediate type.

Key words : age variation, enamel pattern, *Eothenomys smithii*, size of molar, third upper molar.

With regard to the phylogeny of the Arvicolidae, Bauchau and Chaline (1987), and Chaline and Graf (1988), considered that, based on a comparison of molar structures, the occlusal enamel patterns of the third upper molar tended to vary from simple to more complex forms. The genus *Clethrionomys* develops molar roots with advancing age, whereas the genus *Eothenomys* develops no roots. The two genera, however, resemble each other in many other characters of the skull and dental morphology (Hinton 1926, Kaneko 1990, 1992), and in their karyotypes (Yoshida *et al.* 1989). Through the ontogenetic process of *C. glareolus* (Zejda 1960), *C. rufocanus* (Abe 1982) and *E. andersoni* (Miyao 1966, Kitahara 1995), a large proportion of molars changes from complex enamel

patterns to simpler forms.   No age variation was found, however, on the same molar in *E. smithii* (Tanaka 1971).   Tanaka's (1971) results for *E. smithii* may have been biased because of his relatively small sample group of specimens collected during just one period of the year, when fully adult animals may have been absent.

The purpose of this study, therefore, was to reexamine the age variation in both size and enamel pattern of the third upper molars of *E. smithii*, and to compare the results with those of other *Eothenomys* species.

MATERIALS AND METHOS

A total of 99 specimens of *E. smithii* were collected at Minoura, Toyohama District, Kagawa Prefecture, Japan, (34°02′30″N, 133°37′30″E).   Specimens in each of the 12 months were sampled at one period during the years 1977–80 (Kaneko 1989).   The collecting site for this study was less than 50 km from Tanaka's (1971) site on the same island, Shikoku.   Five measurements of the third upper molar and the condylobasal length (CBL) were taken from cleaned skulls, these were: total length (TM3L), anterior length (AM3L), posterior length (PM3L), the width of dentine confluent spaces between the first and second triangles (WDC) and the depth of the third lingual reentrant fold or the posterior loop (DRF, Fig. 1).   Tooth dimensions were measured to the nearest
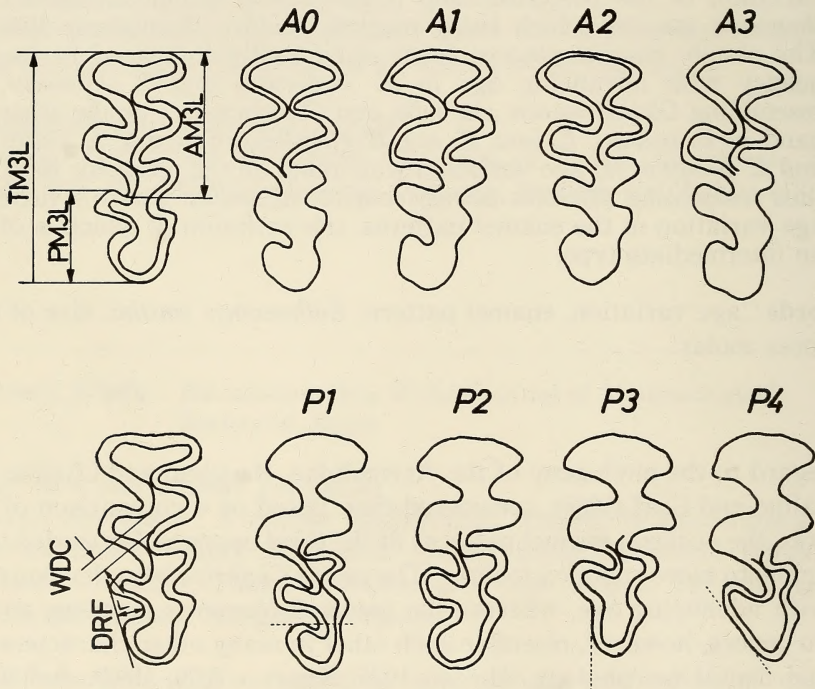

Fig. 1.   Measurements taken (left), and enamel patterns (A0–A3 and P1–P4) on the third upper molar according to Tanaka (1971).   TM3L, AM3L, PM3L, WDC and DRF are explained in text.

0.01 mm using a stereo-microscope (Nikon, SMZ-10) with an objective micro-
meter (Kogaku, minimum interval=0.05 mm). The CBL was measured to the
nearest 0.1 mm with a dial caliper (minimum interval=0.05 mm).

Tanaka (1971) defined the enamel patterns formed on the occlusal surface
by the enamel lamellae only in figures; however, in this study more precise
criteria have been used. Four patterns (A0–A3) in the shape of the dentine
confluent spaces between the second and third triangles were recognized. In
A0, the lamellae do not form two triangles, but a wide dentine confluent space
instead. A1 and A2 are intermediate patterns between A0 and A3 (A1 shows
a smaller protrusion of the enamel lamella, and A2 a larger protrusion). In A3
the lamellae form two complete triangles. Four other patterns (P1–P4) were
observed on the posterior loop, or on the fourth salient angle. In P1 the pattern
is complex, with three reentrant folds on the lingual side, with the third fold
exceeding the transverse line at the anterior edge of the salient angle of the
posterior loop. P2 is intermediate between patterns P1 and P3. P3 has three
salient angles with a straight-sided posterior loop on the lingual side. In P4
the pattern is simple with two reentrant folds on the lingual side and without
concavity on the posterior loop. Enamel patterns were observed on the right
or left molar under a stereo-microscope with a ×20 lens.

In this study, CBL was used as an approximate indicator of age, because it
correlates positively with age as defined by root development in *Clethrionomys
rufocanus* (Kaneko 1990). As there have been no reports indicating sexual
differences in either size or enamel patterns, both males and females were
combined for analysis.

RESULTS

As CBL increased, both total length (TM3L) and anterior length (AM3L)
increased significantly ($r=0.661$, $p<0.001$ and $d. f.=97$ in TM3L; $r=0.676$, $p<$
0.001, $d. f.=97$ in AM3L). As CBL increased, the posterior length (PM3L)
increased until CBL reached 22.5 mm where it reached asymptote, though a
significant regression coefficient was calculated throughout the size of CBL
($r=0.305$, $0.001<p<0.01$, $d. f.=97$, Fig. 2).

The depth of the reentrant fold of the posterior loop (DRF) was nearly
constant against CBL=22–25 mm and decreased slightly in CBLs larger than 25
mm. With the increase of CBL, width of the dentine confluent spaces (WDC)
remained almost constant. Regression coefficients between CBL and DRF,
and between CBL and WDC were insignificant ($r=-0.198$, *n. s.*, $d. f.=97$ in
DRF; $r=-0.057$, *n. s.*, $d. f.=97$ in WDC, Fig. 3).

The average length (X), standard deviation (SD), and coefficient of varia-
tion (CV) of these five measurements are tabulated for five size classes of CBL.
Average TM3L and AM3L increased continuously as CBL increased, whereas
PM3L ceased to increase from the CBL=22.0 mm class onwards. The coeffi-
cient of variation is greater in PM3L than in TM3L and AM3L for each size
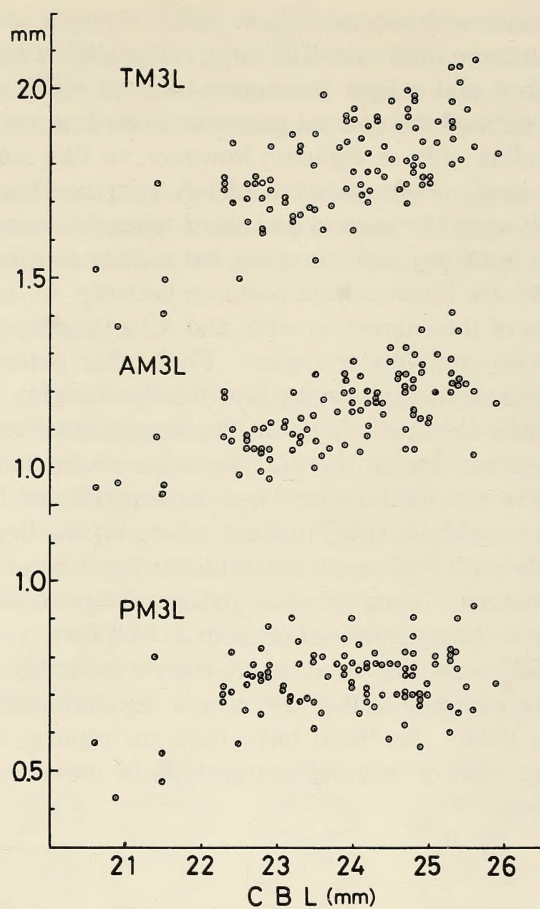class. Average DRF decreased from CBL=25 mm onwards, whereas average

Fig. 2.   Plots of TM3L, AM3L and PM3L against the condylobasal length (CBL).
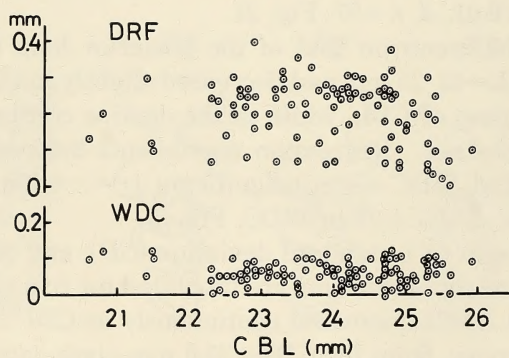

Fig. 3.   Plots of WDC and DRF against the condylobasal length (CBL).

WDC was slightly longer in the 20 mm CBL class. The coefficient of variation was greater was DRF in the 25 mm CBL class (Table 1).

The association of enamel patterns between left and right third upper molars was tested using the G-test with Williams' adjustment (Sokal and Rohlf 1973). No independence was shown between right and left molars in the patterns of the dentine spaces between the second and third triangles (A0-A3 ; $G_{adj} = 35.66$, $p < 0.005$, *d. f.* $= 9$, Table 2), or between right and left patterns of the posterior loop (P1-P4 ; $G_{adj} = 43.88$, $p < 0.005$, *d. f.* $= 9$, Table 2). Similarly no association was shown between the patterns of the dentine confluent spaces and those of the posterior loop (A0-A3 and P1-P4, $G_{adj} = 6.56$, *n.s.*, *d. f.* $= 9$, Table 3). Consequently, the left third upper molar was used for further studies.

In an analysis of the width of dentine spaces (WDC) and the enamel patterns (A0-A3) between the second and third triangles for each of the five size classes of CBL, the 0.05-0.10 mm WDC class and pattern A2 predominated in every CBL size class over 22.0 mm, and average WDC remained almost constant throughout the size classes (Fig. 4). No association was found between the enamel patterns and the five CBL size classes ($G_{adj} = 18.73$, *n.s.*, *d. f.* $= 12$, Table 4). When the frequency of the patterns was divided into three body weight classes, as defined by Tanaka (1971), or by CBL (20.5-, 23.0-, and 25.0- mm

Table 1. Five measurements of the third upper molars of *Eothenomys smithii*.

		Five CBL classes (mm)				
		20.5-	22.0-	23.0-	24.0-	25.0-
N		5	19	21	37	17
Total	*X*	1.508	1.720	1.769	1.832	1.908
length	*SD*	0.150	0.082	0.119	0.093	0.102
(TM3L)	*CV*	9.946	4.757	6.661	5.064	5.368
(mm)	Min.	1.37	1.50	1.55	1.63	1.76
	Max.	1.75	1.86	1.99	2.00	2.08
Anterior	*X*	0.974	1.068	1.122	1.190	1.215
length	*SD*	0.060	0.060	0.073	0.080	0.087
(AM3L)	*CV*	6.181	5.572	6.534	6.679	7.170
(mm)	Min.	0.93	0.97	1.00	1.04	1.03
	Max.	1.08	1.20	1.25	1.36	1.41
Posterior	*X*	0.564	0.730	0.748	0.739	0.752
length	*SD*	0.144	0.071	0.073	0.076	0.087
(PM3L)	*CV*	25.497	9.760	9.818	10.303	11.510
(mm)	Min.	0.43	0.57	0.61	0.56	0.60
	Max.	0.80	0.88	0.90	0.90	0.93
Confluent	*X*	0.094	0.050	0.064	0.056	0.059
width	*SD*	0.026	0.028	0.029	0.032	0.032
(WDC)	*CV*	27.766	55.800	44.479	57.271	54.422
(mm)	Min.	0.05	0.00	0.00	0.00	0.00
	Max.	0.12	0.10	0.11	0.12	0.10
Depth of	*X*	0.170	0.223	0.221	0.217	0.130
reentrant	*SD*	0.082	0.076	0.088	0.083	0.087
fold	*CV*	48.177	34.187	39.638	38.199	67.154
(DRF)	Min.	0.10	0.07	0.06	0.05	0.00
(mm)	Max.	0.30	0.40	0.36	0.38	0.30

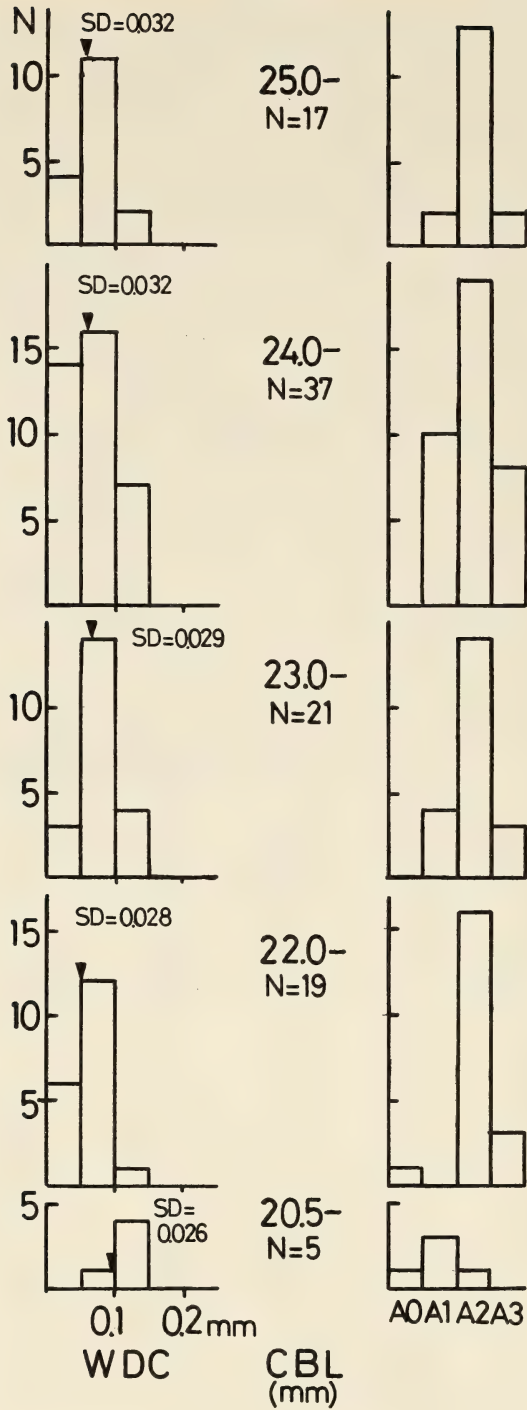Fig. 4. Frequency distributions of WDC measurements and enamel patterns A0–A3 of the dentine space for five CBL classes. A closed triangle shows the average, and *SD* indicates the standard deviation of the average.
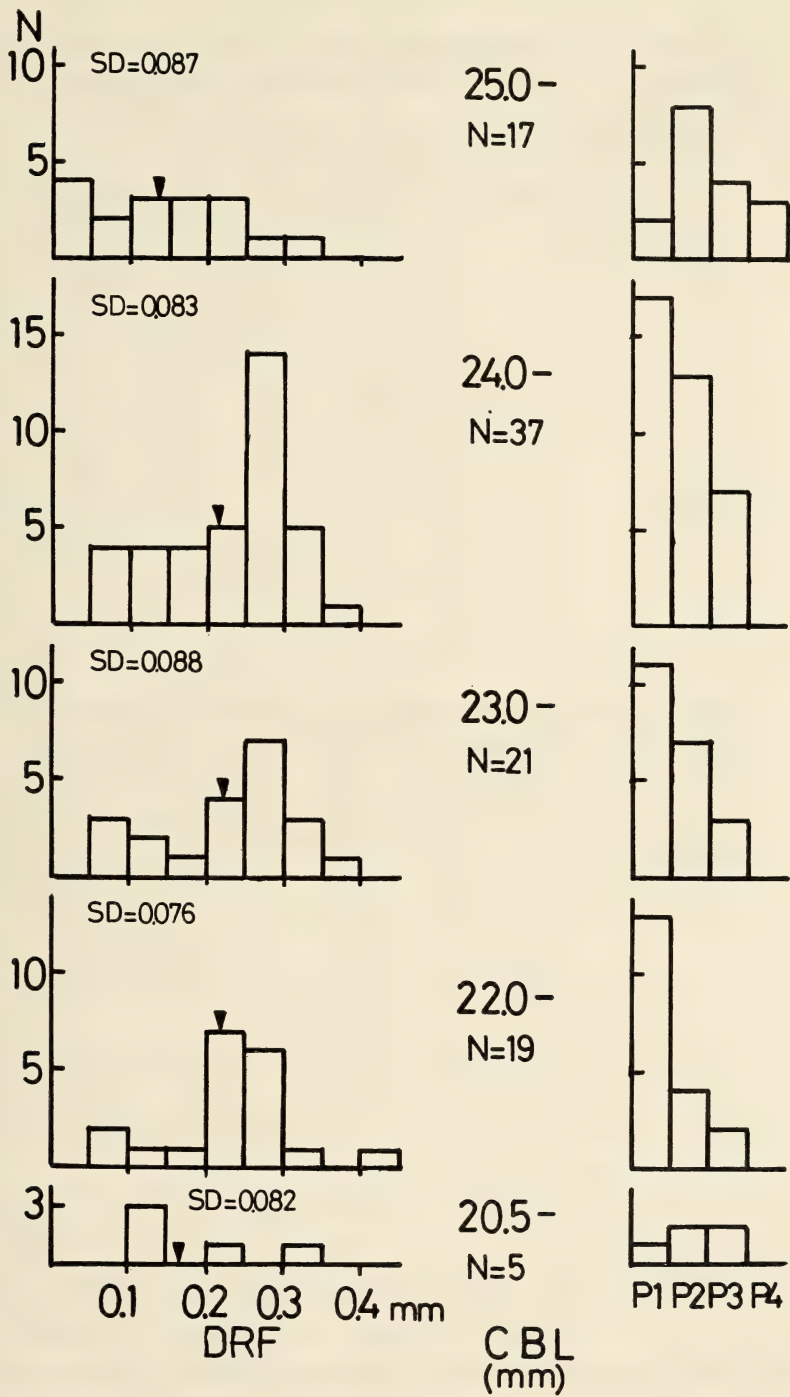
Fig. 5. Frequency distributions of DRF measurements and enamel patterns P1–P4 for five CBL classes. A closed triangle shows the average, and *SD* indicates the standard deviation of the average.

Table 2. A test of independence for frequencies of the enamel patterns (A0–A3 and P1–P4) between the right and left molars of *Eothenomys smithii*.

		The	left			
		Confluent	patterns of	the 2nd	and 3rd	spaces
		A0	A1	A2	A3	Total
The right	A0	1	2	0	0	3
	A1	1	11	2	0	14
	A2	0	6	59	4	69
	A3	0	0	1	12	13
	Total	2	19	62	16	99
		Patterns	of posterior	loop		
		P1	P2	P3	P4	Total
The right	P1	34	7	0	1	42
	P2	10	24	5	0	39
	P3	0	3	12	0	15
	P4	0	0	1	2	3
	Total	54	34	18	3	99

Table 3. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) of *Eothenomys smithii*.

		Confluent patterns	between the 2nd	and 3rd	spaces	
		A0	A1	A2	A3	Total
Posterior	P1	1	5	28	10	44
loop	P2	0	7	24	3	34
patterns	P3	1	6	9	2	18
	P4	0	1	1	1	3
	Total	2	19	62	16	99

based on Table 4), no significant association was shown between the two dimensions ($G_{adj} = 8.13$, *n.s., d. f.* $= 9$ for CBL ; $G_{adj} = 4.83$, *n.s., d. f.* $= 6$ for body weight, Table 5).   Thus, the variation of the pattern of the dentine confluent spaces (A0–A3) is independent of age.

In an analysis of the depth of the reentrant fold (DRF) and of the enamel patterns of the posterior loop (P1–P4) for each CBL size class, patterns P1–P3 appeared with similar frequencies in CBL classes from 22 mm to 24 mm, as did DRF, with almost the same average, though within a wide range between 0.05 and 0.45 mm (Fig. 5).   In the 25 mm CBL class, average DRF became slightly shallower, and pattern P4 appeared for the first time, and P2 became the most frequent pattern.   No association was found between the patterns of the posterior loop and the five CBL size classes at the 5% level ($G_{adj} = 19.36$, $0.05 < p < 0.1$, *d. f.* $= 12$, Table 4).   However, when the patterns of the posterior loop were classified into three body weight classes, as defined by Tanaka (1971), and

Table 4. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) and five CBL size classes of *Eothenomys smithii*.

		Confluent patterns between the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
Five	20.5–	1	3	1	0	5
CBL	22.0–	1	0	15	3	19
classes	23.0–	0	4	14	3	21
(mm)	24.0–	0	10	19	8	37
	25.0–	0	2	13	2	17
	Total	2	19	62	16	99

		Posterior loop patterns				
		P1	P2	P3	P4	Total
Five	20.5–	1	2	2	0	5
CBL	22.0–	13	4	2	0	19
classes	23.0–	11	7	3	0	21
(mm)	24.0–	17	13	7	0	37
	25.0–	2	8	4	3	17
	Total	44	34	18	3	99

Table 5. A test of independence for frequencies between the enamel patterns (A0–A3 and P1–P4) and three body weight classes of *Eothenomys smithii*.

		Confluent patterns between the 2nd and 3rd spaces				
		A0	A1	A2	A3	Total
Body	10.0–	1	3	3	2	9
weight	20.0–	1	12	41	11	65
(g)	30.0–	0	4	18	3	25
	Total	2	19	62	16	99

		Posterior loop patterns				
		P1	P2	P3	P4	Total
Body	10.0–	3	3	3	0	9
weight	20.0–	36	21	8	0	65
(g)	30.0–	5	10	7	3	25
	Total	44	34	18	3	99

by CBL (20.5–, 23.0– and 25.0– mm based on Table 4), significant associations were found for both dimensions ($G_{adj} = 17.69$, $p < 0.01$, $d.f. = 6$, for CBL ; $G_{adj} = 15.34$, $p < 0.025$, $d.f. = 6$, for body weight, Table 5).

When the total (TM3L), anterior (AM3L), and posterior (PM3L) lengths of the third upper molar were examined in relation to the four enamel patterns on the posterior loop (P1–P4) for each of the four CBL size classes (Fig. 6), it was found that average PM3Ls tend to decrease from P1 to P4 at the 24 and 25 mm CBL classes, whereas average AM3L increased slightly.
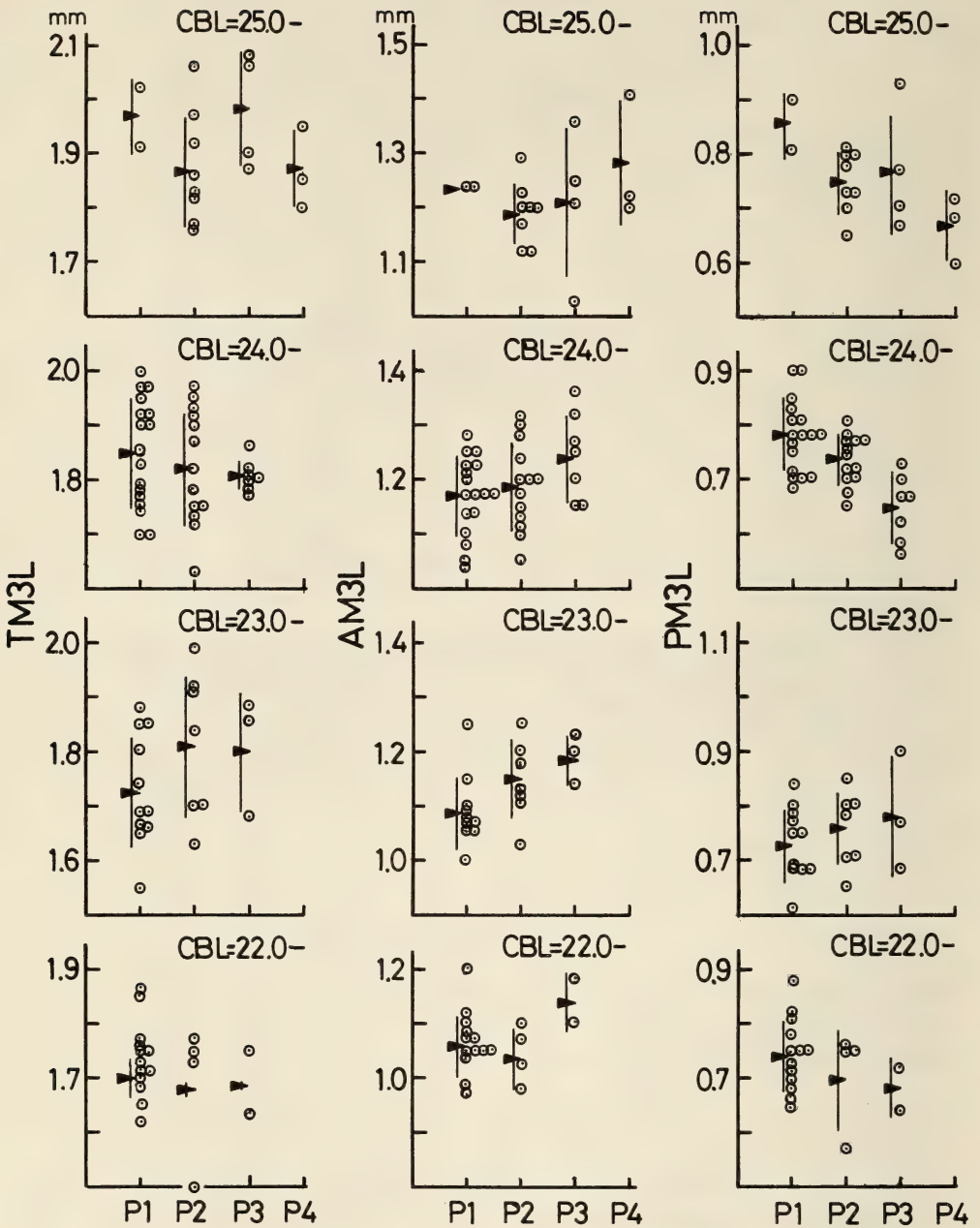
Fig. 6.   Measurements of TM3L, AM3L and PM3L against enamel patterns P1–P4 for four CBL classes.   A closed triangle shows the average, and a vertical line indicates the standard deviation of the average.

DISCUSSION

In revising the taxonomic position of *Eothenomys smithii*, Tanaka (1971) first showed that no age variation was found on the enamel patterns of the dentine confluence between the second and third triangles and the enamel patterns of posterior loop on the third upper molar. Sixty-six specimens used by Tanaka (1971) were collected at the end of July at 1700 m on Mt. Tsurugi, Tokushima Prefecture, Shikoku, Japan. The breeding season of this vole is at its peak during July when Tanaka (1971) collected specimens, and fully adult voles represented only a small proportion of the population (Kaneko and Morii 1976). In this study 99 specimens collected throughout a year were examined, and these included a larger proportion (26%) of old adults with body weight heavier than 30 g, than in Tanaka's (1971) sample (16%). In the specimens collected for this study, however, the posterior loop of the third molar showed some age variation, as shown by the increase of the frequency of P2, the appearence of P4 (the simple form) and the shallower reentrant fold (DRF) in the largest CBL size class (Figs. 3 and 5, and Table 1).

Table 6. The relationship between enamel patterns and skull sizes in species of *Eothenomys* (data from Kaneko 1990, 1992).

Species	Size (mm)	Posterior loop patterns*				
		Type 6	Type P2	Type P3	Type P4	Total
E. shanseius	12.2–	5	0	1	0	6
(I–M3)	13.1–	6	2	3	2	13
$G_{adj}=52.02$	14.0–	1	6	9	3	19
d. f.=12	15.2–	0	2	8	16	26
$p<0.05$	16.1–	0	1	6	10	17
	Total	12	11	27	31	81
E. inez	11.3–	1	0	0	0	1
(I–M3)	12.2–	1	2	4	0	7
$G_{adj}=6.28$	13.1–	0	8	12	0	20
d. f.=9, *n. s.*	14.0–	0	2	3	0	5
	Total	2	12	19	0	33
E. eva	11.3–	0	0	0	3	3
(I–M3)	12.2–	0	3	2	12	17
$G_{adj}=5.74$	13.1–	0	0	9	20	29
d. f.=6, *n. s.*	14.0–	0	0	0	1	1
	Total	0	3	11	36	50
E. regulus	21.0–	1	2	0	0	3
(CBL)	22.0–	1	3	0	0	4
$G_{adj}=8.94$	23.0–	0	5	1	0	6
d. f.=15, *n. s.*	24.0–	0	6	2	0	8
	25.0–	1	5	0	0	6
	26.0–	0	6	2	0	8
	Total	3	27	5	0	35

* Type 6 has three salient angles on the lingual side, a short posterior loop, and a confluent dental isthmus between triangles. Except for Type 6, all types appearing in Kaneko (1990, 1992) were followed in the present classification.
n.s. : non-significant.

A statistically significant association was found between the posterior loop patterns (P1–P4) and three CBL classes (20.5-, 23.0- and 25.0- mm, Table 4), because the DRF was nearly constant throughout the 22–24 mm CBL classes but decreased only in the largest 25 mm CBL class (Fig. 3 and Table 1).   Furthermore, a test between patterns P1–P4 and the three body weight classes would be significant (Table 5), because body weight correlates siginificantly with CBL ($r = 0.911$, $p < 0.001$, $d.f. = 97$) and individuals with a CBL of more than 25 mm correspond with those of body weights of over 30 g.

As age increases, the pattern of the posterior loop tends to become simple in *Clethrionomys glareolus* (Zejda 1960) and *C. rufocanus* (Abe 1982), which have rooted molars in older individuals.   The simple form increases in frequency from the root ratio exceeding 63% in *C. glareolus* and 32% in *C. rufocanus* (Zejda 1960, Abe 1982).   Due to the loss of the third reentrant fold with age, the proportion of the simple form increases in *C. glareolus* and *C. rufocanus*.   In contrast, age variation was not observed on the loop in *Microtus pennsylvanicus*, which remains rootless throughout life (Oppenheimer 1965).

Among ten species of *Eothenomys* having rootless molars throughout life, a gradual transition is found in the age variation of the posterior loop pattern : in *E. andersoni* the ratio of the simple form of the posterior loop increases with advancing age (Miyao 1966, Kitahara 1995), and in *E. shanseius* the proportion of the simple form increases with increasing CBL, though samples from different populations were pooled (Table 6), resembling in this respect *Clethrionomys glareolus* and *C. rufocanus*.   In contrast, age variation has not been observed in either *E. inez, eva, regulus, chinensis, wardi, custos*, or *proditor*, they thus resemble *Microtus pennsylvanicus*, though samples from different populations were pooled (Table 6 and unpublished data).   Thus, *E. smithii* is an intermediate type between these two groups showing a little age variation.

In *E. smithii*, the posterior length of the third upper molar (PM3L) ceased to grow with age, though both the total molar length (TM3L) and the anterior length (AM3L) increased with age (Table 1 and Fig. 2).   The growth of TM3L, therefore, is related to that of the anterior length.   However, when PM3L was plotted against the four posterior loop patterns (P1–P4, Fig. 6), the posterior length tended to become relatively shorter from P1 to P4 as CBL increases.   In *C. rufocanus*, the simple form (P4) increases greatly in frequency (Abe 1982), as the length decreases with advancing age (Abe 1973).   It is suggested, therefore, that the increase in the frequency of P4 is due to a shortening of PM3L with advancing age.   Furthermore, in *E. smithii* it appears that PM3L does not decrease prominently with advancing age (Fig. 2), because the increase in the frequency of the simple P4 pattern is relatively lower than in *C. rufocanus*.

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Phylogenetic implications of variations in rDNA and mtDNA in red-backed voles collected in Hokkaido, Japan, and in Korea

Shigeharu WAKANA[1], Mitsuru SAKAIZUMI[2], Kimiyuki TSUCHIYA[3], Mitsuhiko ASAKAWA[4], Sang Hoon HAN[5], Keisuke NAKATA[6] and Hitoshi SUZUKI[7]*

[1]*Department of Genetics, Central Institute for Experimental Animals, Nogawa, Miyamae-ku, Kawasaki 213,* [2]*Department of Environmental Science, Faculty of Science, Niigata University, Niigata 950-21,* [3]*Experimental Animal Center, Miyazaki Medical College, Kiyotake, Miyazaki 889-16,* [4]*Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069,* [5]*Institute of Applied Zoology, Faculty of Agriculture, Hokkaido University, Sapporo 060,* [6]*Hokkaido Forest Experiment Station, Bibai, 079-01,* [7]*Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Kita 10, West 5, Kita-ku, Sapporo 060, Japan*
(*To whom correspondence should be addressed)
Fax. 011-706-2225, E-mail. htsuzuki @ eesbio. hopudai. ac. jp

Abstract. Restriction fragment length polymorphisms (RFLPs) in nuclear ribosomal DNA (rDNA) spacers and mitochondrial DNA (mtDNA) were examined in red-backed voles collected in Hokkaido (Japan), and Korea. These voles have been classified into six species on the basis of morphological characteristics, such as dental morphology. The RFLPs of the rDNA allowed us to classify the voles into three distinct groups : rCrt (*Clethrionomys rutilus*), rCrf (*C. rufocanus, C. sikotanensis* and *Eothenomys regulus*) and rCrx (*C. rex* and *C. montanus*). The estimated sequence divergence between rCrt and rCrf and that between rCrf and rCrx were 4.8% and 2.3%, respectively. In the rCrf group, no major differences in mtDNA were observed among the populations from the mainland of Hokkaido, Rishiri Island, and Daikoku Islet. Similarly, in the rCrx group, mtDNA haplotypes from the mainland of Hokkaido and Rishiri I. were closely related each other, indicating that there have been genetic exchanges between the populations after speciation, or those haplotypes are derived from recent common origin. The Korean red-backed vole, which is sometimes referred to *E. regulus*, had rDNA identical to that of the rCrf group from Hokkaido. By contrast, the mtDNA haplotype of the Korean vole was substantially different from that of *C. rufocanus* in Hokkaido (8% sequence divergence). These results imply that the Korean red-backed vole and *C. rufocanus* in Hokkaido are very closely related and that ancestrally diverged mtDNA haplotypes have been maintained in the different geographic regions.

Key words. *Clethrionomys,* mitochondrial DNA, restriction fragment length polymorphism, ribosomal DNA, the red-backed vole.

Red-backed voles, which are small rodents that live in the fields and mountains of the Palaearctic Region, are extremely complicated in terms of taxonomy. Red-backed voles have been traditionally classified into two genera, *Clethrionomys* and *Eothenomys*, on the basis of differences in morphological characteristics, such as the presence or absence of rooting of the molars (Hinton 1926). However, it is uncertain whether or not such criteria are phylogenetically appropriate.   In terms of morphological criteria, the red-backed voles living in Hokkaido, Japan, are classified as *Clethrionomys* because of the presence of rooting of the molars.   On the mainland of Hokkaido, three different forms of vole are known : the Northern red-backed vole (*C. rutilus* or *C. r. mikado*) ; the gray red-backed vole (*C. rufocanus* or *C. r. bedfordiae*) ; and a recently identified form, represented by *C. rex* and *C. montanus* (Imaizumi 1971, 1972). Morphologically, *C. rufocanus* and *C. rex* are similar and some taxonomists question the classification of *C. rex* as a distinct species (Aimi 1980, Corbet 1978, Musser and Carleton 1993).   Another taxonomic issue is the classification of voles on the peripheral islands of Hokkaido, and on Rishiri Island in particular, where the existence of two different forms of the red-backed vole, namely, *C. sikotanensis* and *C. rex*, have been reported (Imaizumi 1971).   *C. sikotanensis* has, however, been considered as synonymous with *C. rufocanus* by Abe (1984) and Kaneko and Sato (1993).   According to Imaizumi (1972), *C. rex*-like voles on the mainland should be designated *C. montanus*, a species different from *C. rex* on Rishiri I.   However, Abe (1984) considered that *C. rex* and *C. montanus* are synonymous.   It is of interest, moreover, that voles living on Daikoku Islet, adjacent to the southeastern coast of Hokkaido, are usually classified as *C. rufocanus* but display morphological characteristics of both *C. rufocanus* and *C. rex* (Abe 1984).   Therefore, the unequivocal taxonomic classification of the voles on Daikoku I. has not yet been made (Abe 1984).

The Korean red-backed vole provides another intriguing question with respect to classification.   It has been included in the genus *Clethrionomys* and classified as a species, *C. regulus*, that is endemic to Korea (Corbet 1978).   In a detailed morphological study of voles from Russia and Korea, Kaneko (1990) showed that all of the examined specimens from Korea had rootless teeth, in contrast to the individuals from Russia and have subsequently been classified as *Eothenomys regulus* (Corbet and Hill 1991).   The true geographical demarcation line between *C. rufocanus* and *E. regulus* lies on the western and southern boundary of the Kaima Plateau, North Korea (Kaneko 1990).

Morphological studies have not provided sufficient information about the classification of red-backed voles and a single morphological characteristic, such as rooting of molars, is insufficient for classification of a given species. Karyological studies have been made on some members of the complicated genera of *Clethrionomys* and *Eothenomys*.   However, the karyotypes appear to be very similar and no informative variations have been reported (Kashiwabara and Onoyama 1988, Tsuchiya 1981, Yoshida *et al*. 1989).

Both rDNA and mtDNA provide powerful diagnostic markers for the identification of populations because 1) both mtDNA and rDNA exist as

multiple copies in the mammalian genome and, thus, are easily analyzed, and 2) many RFLPs are specific to each population.   Combined analyses, exploiting both cytoplasmic and nuclear markers, should provide much more reliable information on the timing of divergence and the topology of the phylogenetic tree among populations of given animal species.

In the present study, we compared variations in rDNA and mtDNA among six morphologically different forms of red-backed voles collected on the mainland of Hokkaido, on Rishiri I., on Daikoku I. and in Korea.   Here we demonstrate that the six taxa can be classified as three distinct species.

MATERIALS AND METHODS

1. Voles

A total of 36 voles (Table 1), collected from eight localities (Fig. 1), was used for the analysis of rDNA and mtDNA.

2. Blot analysis

Nuclear DNA was prepared from the liver of each vole as described by Maniatis *et al.* (1982).   Southern blot analysis was carried out as described by Suzuki *et al.* (1994b).   DNA was digested with eight restriction enzymes for the analysis of mtDNA (*Aat*I, *Apa*I, *Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII, *Pst*I and *Xba*I) and with ten restriction enzymes for the analysis of rDNA (*Aat*I, *Bam*HI, *Bgl*II,

Table 1.   List of samples used and specific types of morphology, rDNA and mtDNA.

Serial no. and locality	Name of species, as typed with morphological characteristics	rDNA repetype	mtDNA haplotypes (no. of individuals with common haplotypes)
Hokkaido, Japan			
1. Bekkai	*C. rutilus*	rCrt	mCrt1 (1)
2. Teshio	*C. rufocanus*	rCrt	mCrf1 (4)
	C. montanus	rCrx	mCrx1 (4)
3. Tobetsu	*C. rufocanus*	rCrf	mCrf1 (2), mCrf2 (5)
			mCrf3 (1)
4. Naganuma	*C. rufocanus*	rCrf	mCrf1 (1), mCrf2 (2)
			mCrf4 (3)
5. Ohtaki	*C. rufocanus*	rCrf	mCrf3 (1)
6. Rishiri I.	*C. sikotanensis*	rCrf	mCrf5 (1)
	C. rex	rCrx	mCrx2 (1)
7. Daikoku I.	*C. rufocanus*	rCrf	mCrf1 (8)
Korea			
8. Mt. Chiri	*E. regulus*	rCrf	mErg1 (2)

Fig 1. Localities at which red-backed voles were sampled.   Numbers assigned to localities are the same as in Table 1.

*Dra*I, *Eco*RI, *Hind*III, *Pst*I, *Pvu*II, *Sac*I, and *Xba*I).   The digested DNAs were immobilized on nylon filters and then allowed to hybridize sequentially with ^{32}P-labeled probes of rDNA, namely, 28S, 18SB, and INT (Suzuki *et al.* 1994b). A mtDNA probe used was the whole mtDNA genome that was prepared from the liver of a hamster, as described by Wakana *et al.* (1986).   After hybridization, filters were washed twice with 2 × SSC (0.3 M NaCl-0.03 M Na citrate solution with pH 7.0) containing 0.1% (w/v) sodium dodecyl sulfate at room temperature.   Autoradiographs were obtained either by exposing hybridized membranes to X-ray film or with an image analyzer (Bio Image Analyzer, BAS2000, Fuji Film, Japan).

3. Construction of restriction maps of the rDNA

From the patterns of hybridization after single digestions, restriction maps were constructed for the coding and internal spacer regions of rDNA because most restriction sites in the coding region as well as a *Dra*I site in the internal spacer were conserved. By reference to restriction-site maps of the coding and the internal spacer regions, the location of the restriction sites on the external spacer region, which flanked the genes for 18S and 28S rRNA, was estimated by single digestion and hybridization with the 18SB and 28S probes. Since the probes were localized to the distal end of the coding regions, only the restriction sites nearest to the distal end of the genes for 18S or 28S rRNA could be mapped. Although length polymorphisms within the genome were observed in certain regions of the external spacer in most samples investigated, only the most prominent bands were taken into account for construction of the physical maps.

4. Construction of phylogenetic trees

To estimate the sequence divergence among the three major rDNA repetypes we compared the arrangement of restriction sites between pairs of repetypes and counted the number at common and different sites. The sequence divergence among haplotypes of mtDNA was estimated from the number of common and different restriction fragments observed. Employing a method developed by Gotoh *et al.* (1979), in which backward mutations and parallel mutations are taken into account, we produced a matrix of sequence divergences among all possible combinations of rDNA repetypes (Table 2) and mtDNA haplotypes (Table 3). Then we constructed phylogenetic trees using the unweighted pair-group (UPGMA) method (Sokal and Michener 1958) and the neighbor-joining (NJ) method (Saitou and Nei 1987).

Table 2. Sequence divergence among the rDNA repetypes (upper right), based on the number of common and different restriction sites (lower left).

		Sequence divergence (%)		
		rCrt	rCrx	rCrf
rCrt		—	6.4	4.8
rCrx	28S*	6/4**		2.3
	18S	5.5/4.5	—	
	INT	1/3		
	total	12.5/11.5		
rCrf	28S	6/4	8/2	—
	18S	6.5/3.5	8/2	
	INT	2.5/2.5	2.5/1.5	
	total	15/10	18.5/5.5	

* The external (28S, 18S) spacer region and the internal spacer and coding regions (INT).
**Number of sites (common/different).

Table 3. Sequence divergence among the mtDNA haplotype (upper right), based on the number of common and different restriction fragments (lower left).

Haplo-type	Sequence divergence (%)								
	mCrt1	mCrx1	mCrx2	mCrf1	mCrf2	mCrf3	mCrf4	mCrf5	mErg1
mCrt1	—	11.7	11.8	8.3	8.3	10.3	8.4	8.4	11.3
mCrx1	5/49*	—	0.3	7.5	6.8	7.5	8.6	6.8	7.1
mCrx2	5/50	26/3	—	7.8	6.1	7.8	8.8	7.6	8.2
mCrf1	8/43	8/37	8/39	—	0.3	0.7	0.3	0.7	7.1
mCrf2	8/43	9/36	11/37	25/3	—	1.1	0.3	0.7	8.3
mCrf3	6/47	8/37	8/39	24/6	25/3	—	0.7	0.7	7.1
mCrf4	8/44	7/40	7/42	26/3	24/3	24/6	—	0.7	7.3
mCrf5	8/44	9/36	8/38	24/6	22/6	22/6	24/6	—	7.9
mErg1	5/46	8/34	7/37	8/34	7/38	8/34	8/35	7/35	—

*Number of fragments (common/different).

RESULTS

We analyzed rDNA from 36 individual voles, namely, 34 from Hokkaido and two from Korea. The voles represented all six possible different morphological forms, as listed in Table 1. The analysis revealed that there were three major rDNA repetypes (rCrt, rCrf and rCrx) and nine different mtDNA haplotypes (mCrt1, mCrx1, mCrx2, mCrf1, mCrf2, mCrf3, mCrf4, mCrf5 and mErg1) among the voles examined. The restriction maps of the three repetypes of rDNA are presented schematically in Fig. 2. Although most of the mutations appeared to have been fixed within populations, some mutations were not fixed within a genome. The restriction sites that showed such intragenomic polymorphisms are marked on restriction maps with asterisks. In this study, we counted substantial heterogeneous variation at a particular site as one half of a common site and one half of a different site. Minor bands on the blotting patterns were ignored.

On the basis of the extent of sequence divergence (Tables 2 and 3), we constructed phylogenetic trees for the mtDNA haplotypes and rDNA repetypes by the UPGMA method (Fig. 3). We also constructed phylogenetic trees by the NJ method and obtained trees with topology essentially identical to those presented in Fig. 3. The trees revealed new criteria for classification of the voles. The rDNA and mtDNA trees indicated that *C. rutilus* (rCrt/mCrt1) diverged first from the others. Two lineages of *C. rufocanus* (rCrf/mCrf1-5) and *C. rex* (rCrx/mCrx1, 2) then diverged. Interpopulational differentiation has been low in both cases.

The mtDNA haplotype of the Korean vole (mErg1) was substantially different (8% sequence divergence calculated from Table 3) from those of *C. rufocanus* from Hokkaido (mCrf1, mCrf2, mCrf3, mCrf4 and mCrf5), even though individuals from Korea and Hokkaido had the same rDNA repetype rCrf.
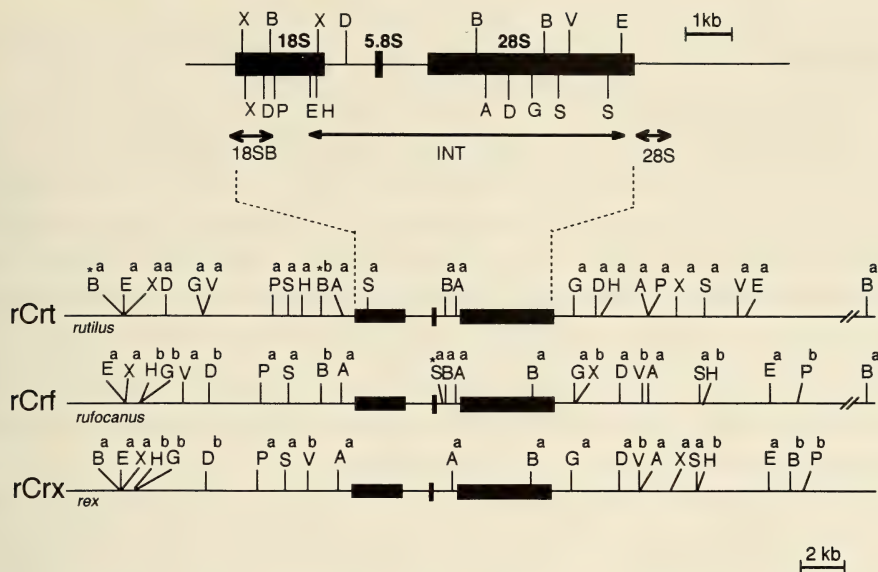
Fig. 2.   Restriction maps of the major rDNA repetypes of *Clethrionomys rutilus* (rCrt), *C. rufocanus* (rCrf), and *C. rex* (rCrx).   With respect to the restriction sites on the flanking spacers, only those nearest to the distal end of the genes for 18S or 28S rRNA are shown. The top diagram shows the conserved restriction sites in the coding and the internal spacer regions of the genes for 18S and 28S RNA (see text), which are not represented in the lower maps.   Positions of probes are also shown by arrows.   Small characters represents types of restriction sites identified after a comparison of restriction maps.   Asterisks indicate polymorphic sites within the genome of a given species.   A, *Aat*I ; B, *Bam*HI ; D, *Dra*I ; E, *Eco*RI ; G, *Bgl*II ; H, *Hind*III ; P, *Pst*I ; S, *Sac*I ; V, *Pvu*II ; X, *Xba*I.
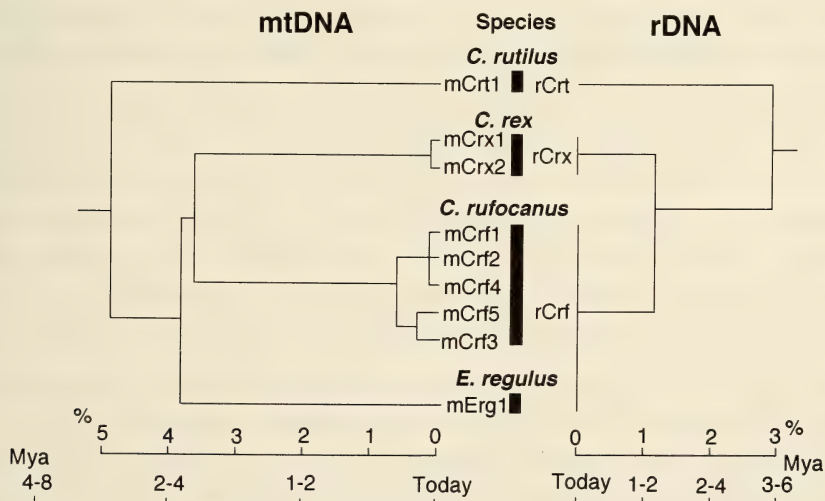
Fig. 3.   Phylogenetic trees for the mtDNA haplotypes and major rDNA repetypes constructed by the UPGMA methods.   Abbreviations are the same as in Table 1.   Divergence time were estimated by assuming the rate of evolution to be 2-4% per Myr for mtDNA RFLPs and 1-2% per Myr for rDNA RFLPs.

DISCUSSION

In the present study, we obtained a new perspective on the phylogeny of red-backed voles in Hokkaido and Korea.   Our data indicate that three true species of red-backed voles exist in Hokkaido, namely, *C. rex*, *C. rufocanus* and *C. rutilus*.   The two morphological forms of the red-backed voles reported by Imaizumi (1971, 1972), namely, *C. rex* from Rishiri I. and *"C. montanus"* from the Hidaka Mountains, Hokkaido, can be regarded as synonymous species, as described by Abe (1984).   Similarly, we found that *"C. sikotanensis"* from Rishiri I. (Imaizumi 1971) and voles from Daikoku I. were very closely related to *C. rufocanus* from the mainland of Hokkaido on the basis of rDNA and mtDNA sequences.   Thus *"C. sikotanensis"* from Rishiri I. and the voles from Daikoku I. can all be regarded as *C. rufocanus*.

The Korean red-backed vole collected on Mt. Chiri, which was a member of a species that is usually known as *E. regulus* (Corbet and Hill 1991), had the same rDNA RFLP profile as individuals collected in Hokkaido with respect to the 24 restriction sites that were investigated.   Since the rDNA RFLP exhibits similar patterns within the same reproductive population and distinct patterns when different reproductive populations are compared (Arnheim *et al.* 1980, Coen *et al.* 1982, Suzuki *et al.* 1986, 1987, 1990, 1994a, 1994b), these data indicate that the Korean red-backed vole is closely related phylogenetically to *C. rufocanus*.   The absence of rooting of the molars in the Korean vole (Kaneko 1990, 1992) is a characteristic that may have developed within a short period of evolutional time in the Korean population.   Although the Korean red-backed vole has been classified as *Eothenomys* on the basis of its dental characteristics (Hinton 1926), it is closely related to *C. rufocanus*.   Hinton's criterion, whereby voles are classified into different genera on the basis of rooting of molars, may include such exception or itself be inappropriate.

The mtDNA haplotype of the Korean vole was substantially different from those of *C. rufocanus* from Hokkaido, Japan (Fig. 3).   Since the major rDNA repetype was identical for the Korean vole and the populations in Hokkaido, the existence of a distinct mtDNA haplotype might be due to the difference in the mode of inheritance between rDNA and mtDNA.   Such phenomena are frequently observed in many organisms, including mammalian species (e.g ; Ferris *et al.* 1983, Tegelström 1987, Yonekawa *et al.* 1988).   For example, in the case of Japanese house mice, the major genetic elements are those of *Mus musculus musculus,* which were introduced from the Asian continent, and the minor genetic elements are those of *M. m. castaneus,* which is thought to have existed on the Japanese islands in ancient times.   In Hokkaido and the northern part of Honshu, *castaneus*-type mtDNA exists dominantly as a relict component (Yonekawa *et al.* 1988).   In the case of the Korean red-backed vole described in this study, the difference in the rDNA and mtDNA phylogenies may have been due to the maintenance of ancestrally diverged mtDNA molecules in the different geographic area, Korean Peninsula and Hokkaido.   The

timing of the divergence of mtDNAs of the Korean vole and *C. rufocanus* from Hokkaido (approximately 8% sequence divergence, Table 3) is estimated to be 2-4 million years ago (Mya), assuming that the rate of evolution of mtDNA to be 2-4% per 1 million years (Myr) (Wilson *et al.* 1985). The timing of the divergence corresponds to that of the divergence of *C. rufocanus* and *C. rex* (Fig. 3).

The sequence divergence between the rDNA repetypes of *C. rufocanus* and *C. rex,* rCrf and rCrx, was 2.3% (Table 2) and the timing of divergence between the two repetypes was estimated to be 1.2−2.4 Mya on the assumption that the rate of evolution of the spacers of rDNA has been 1−2% per 1 Myr (Hosoda *et al.* 1993, Suzuki *et al.* 1994b). The timing of the divergence of mtDNAs of *C. rufocanus* and *C. rex* (8% sequence divergence, Table 3) is estimated to be 2−4 Mya, as mentioned above. Thus, it is suggested that dispersal and species differentiation occurred in the ancestral populations of *rufocanus/rex* about 2 Mya, at the beginning of the glacial age. The sequence divergence between the repetypes of rCrf/rCrx and rCrt was about 6% (Table 2 and Fig. 3) and the timing of divergence between the two group was estimated to be 3−6 Mya. Analysis of mtDNA also support the estimation of time of the *rufocanus/rex-rutilus* split (Fig. 3).

We now propose the following process of species differentiation. (1) The early expansion of red-backed voles in the Palaearctic Region occurred 2.5−5 Mya (see Fig. 3). At that time, *C. rutilus* was diverged from the lineage of *rufocanus/rex*. (2) About 1−2 Mya, the lineage of *rufocanus/rex* differentiated into two species. If *C. rex* is endemic to Hokkaido, the ancestral population that migrated to Hokkaido at that time was the founder population of *C. rex*. (3) In the following glacial period, *C. rufocanus* moved to Hokkaido from the Asian continent. Finally, 0.01−0.02 Mya, when Hokkaido and the continent were last joined with a land bridge (Japan Association for Quarternary Research 1987), populations of *C. rufocanus* communicated genetically among the mainland of Hokkaido and peripheral islands, and the continent. Similarly, populations of *C. rex* on the mainland of Hokkaido and Rishiri I. also communicated genetically at that time.

The evolutionary history of the Korean red-backed vole, *E. regulus,* is currently unknown, but is likely to be well correlated to those of *C. rufocanus*. Molecular identification with various markers will clarify the extent of genetic interchanges between the Korean vole and *C. rufocanus*.

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Home range of female sika deer *Cervus nippon* on Nozaki Island, the Goto Archipelago, Japan

Akira ENDO and Teruo DOI

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan
Fax. 092-642-2645, E-mail. aendoscb @ mbox. nc. kyushu-u. ac. jp

Abstract. The home ranges and habitat preferences of female sika deer (*Cervus nippon*) on Nozaki Island, in the Goto Archipelago were studied by radio-tracking. Six radio-tagged females were tracked continuously during June, August, October and December 1991. Female deer remained in small home ranges including both open and forest habitats throughout the year. These ranges overlapped to a considerable extent, however, individuals moved independently of each other. The females tended to select open habitats from spring to autumn and forest habitats in winter.

Key words : dynamic interaction, female sika deer, habitat preference, home range, radio-tracking.

Among the Cervidae, intraspecific variation in social systems has been found in species which have extensive geographical distributions. This has previously been discussed in the context of the differences in their habitat preferences (Langbein and Thirgood 1989). The sika deer (*Cervus nippon*) occurs very widely in Japan, from the cool temperate zone of Hokkaido in the north to the subtropical zone in the Nansei Shoto in the south, and exhibits considerable clinal variation in body size from north to south (Ohtaishi 1986). Intraspecific variation in male mating tactics have also been among populations of sika deer (Miura 1986). According to Davies (1991), it is likely that the variation in spatio-temporal dispersion of female sika deer may affect the intraspecific variation in male mating tactics. Many previous studies have reported on female home ranges in the cooler northern and central parts of Japan (Miura 1977, Maruyama 1981, Shigematsu *et al*. 1994, Yabe 1994), but none have been made so far in the warm temperate zone of southern Japan.

In the present study, we describe the seasonal changes in size and spacing patterns of home ranges, and the "dynamic interaction" (Macdonald *et al*. 1980) between individual female sika deer on Nozaki Island, in the Goto Archipelago.

STUDY AREA

Nozaki Island is a small (740 ha) island situated in the Goto Archipelago, west of Nagasaki Prefecture (33°10′N, 129°8′E), Kyushu. Most of the island is

covered with secondary evergreen broad-leaved forests dominated by *Castanopsis cuspidata, Camellia japonica* and *Machilus thunbergii*.   The remainder is covered with young plantations of *Pinus thunbergi*, bushes of *Glochidion obovatum* or semi-natural *Miscanthus sinensis, Imperata slyndorica* and *Zoysia japonica* grasslands (Kawahara 1983).   About 700 deer live on the island (Doi and Endo 1992), varying in density from 0.6/ha in forest to 3.1/ha in open grassland.   No hunting or predation occurred during the study period.   Home ranges of female sika deer were studied at the Nozaki site (about 30 ha) in the central part of the island, where the density of deer was highest.   About 40 females utilized this area.

METHODS

In 1991, we captured six female deer using bag net traps (Doi *et al.* 1986) and attached radio neck-collars (50MHz, weight 50 g, ALKITEC Co. Ltd.). Radio-fixes on females were obtained by triangulation with a portable receiver (FT-690, YAESU MUSEN Co. Ltd.), and one or two additional fixes were regularly taken from other points to ensure accuracy.   Radio-fixes, dates and times were all plotted on a 1 : 2500 map.   Tracking in 1991 was carried out in June (early summer, parturition season), August (mid-summer, milking-season), October (autumm, rutting season) and December (winter).   Tagged deer were radio-fixed at three hour intervals for several days.   Since cumulative home range sizes were saturated by the fourth to seventh day, tracking was terminated on the seventh day.   The radio-collar on deer F1 fell off before December 1991, thus data was only collected for deer F2 to F6 during December. The home range sizes were calculated using the convex polygon method (Mohr 1947).   Seasonal shifts in range use were expressed by the degrees of range overlap (*RO*) between two seasons.   It was calculated as :

$$RO = \frac{\text{size of range overlap between two months (ha)}}{\text{home range size (ha)}}$$

The percentage overlap of two home ranges is most useful for identifying spatial distribution (Macdonald *et al.* 1980).   It does not, however, indicate the utilization distribution within the shared parts of overlapping ranges (Doncaster 1990).   This aspect can be elucidated by testing for the dependency in the simultaneous movements of a pair of individuals (dynamic interaction). Analyses of dynamic interactions between females indicate whether two females are more (positive dynamic interaction) or less (negative dynamic interaction) likely to maintain a certain separation given the configuration and utilization of their home ranges (Doncaster 1990).   To test dynamic interaction, a nonparametric comparison was made between the observed distribution of separations between N paired fixes (taken from each animal simultaneously or within 30 minutes of each other), and an expected distribution based on all possible combinations (N²) of the fixes (Doncaster 1990).   A critical separation is chosen within which presence of dynamic interaction is of interest, such as

the furthest separation at which two females could be aware of each other. Since we have no information about the sensitive distance for sika deer, we determined the critical separation as 20 m based on observations of white-tailed deer (*Odocoileus virginianus*, Schwede *et al.* 1993). Expected and observed numbers of paired separations <20 m were compared using the χ^2 - test ($p < 0.05$). When observed numbers of paired separations <20 m was significantly greater than expected, it indicates that those individuals tended to move simultaneously.

In examining habitat preference, the study area was classified into forest and open habitat types. "Forest" includes secondary evergreen broad-leaved forests and young pine plantations in old crop fields, and "open" includes young *Glochidion obovatum* bushes in old crop fields, and grasslands dominated by *Zoysia japonica* in old crop fields and abandoned rice fields.

Habitat selection was expressed by Ivlev's electivity index (E_i; Ivlev 1961). This index was calculated as:

$$E_i = (r_i - N_i)/(r_i + N_i)$$

where r_i is the proportion of the size of the ith habitat type to home range size in each season, and N_i is the proportion of the size of the ith habitat type to the annual home range size.

RESULTS

1. Size and Spatial Distribution of Female Home Ranges

Mean home range sizes ranged from 3.0 to 3.6 ha, and were not significantly different between seasons (Friedman's test: $\chi^2 = 0.360$, $p = 0.948$, see Table 1). Ranges did not shift seasonally (Fig. 1). The rate of overlap was more than 0.5 and there was no significant difference between seasons (Friedman's test: $\chi^2 = 9.4$, $p = 0.585$, Table 2).

Table 1. Seasonal changes in home range size of female sika deer on Nozaki Island. Number of females shown in parenthesis.

Month	Home range size(ha) Mean $\pm$ *SD* (N)
Jun.	$3.47 \pm 0.57\,(6)$
Aug.	$3.27 \pm 1.25\,(6)$
Oct.	$3.03 \pm 0.99\,(6)$
Dec.	$3.60 \pm 0.33\,(5)\,^*$

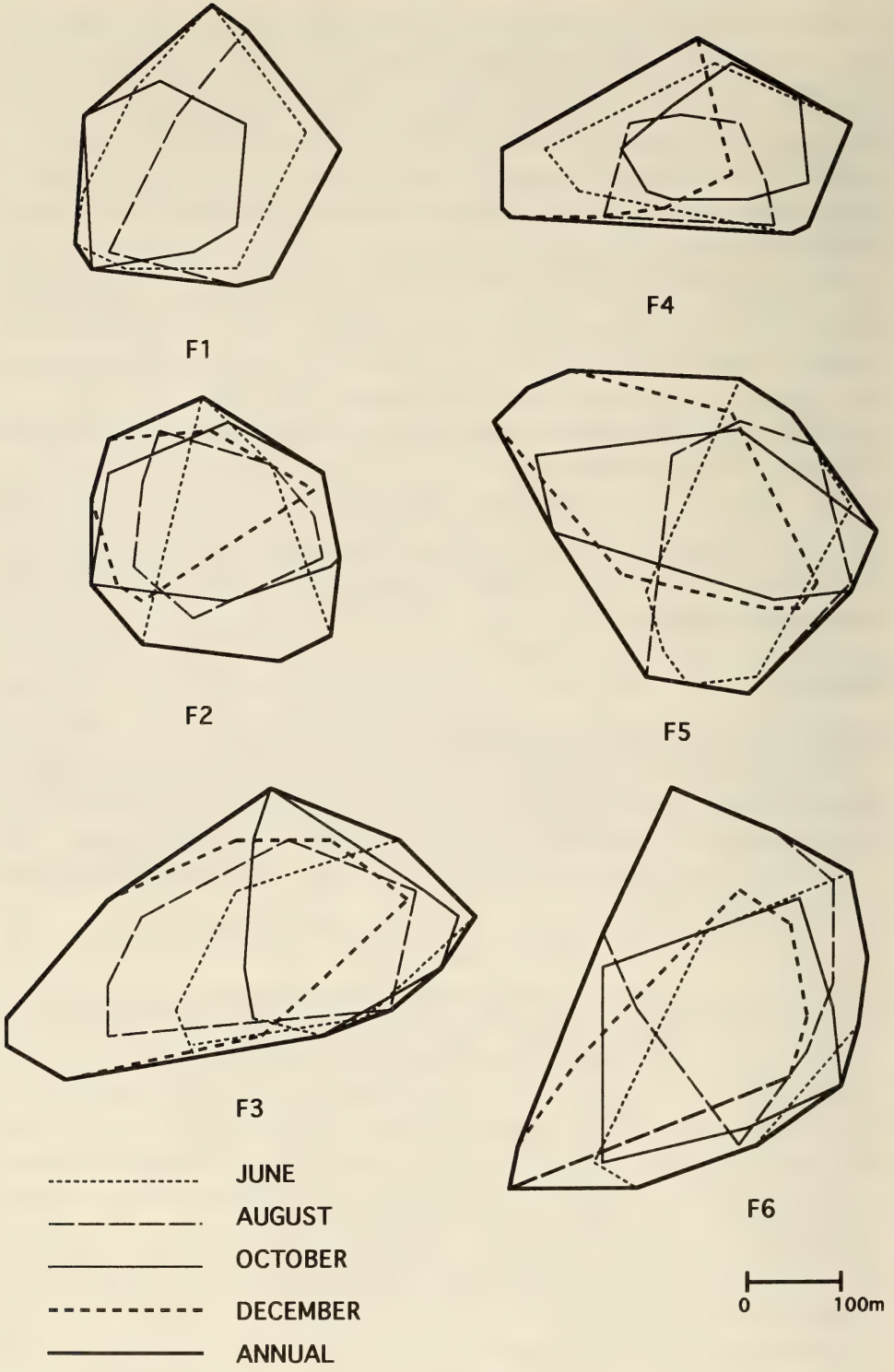*radio-collar of F1 fell off before December 1991.

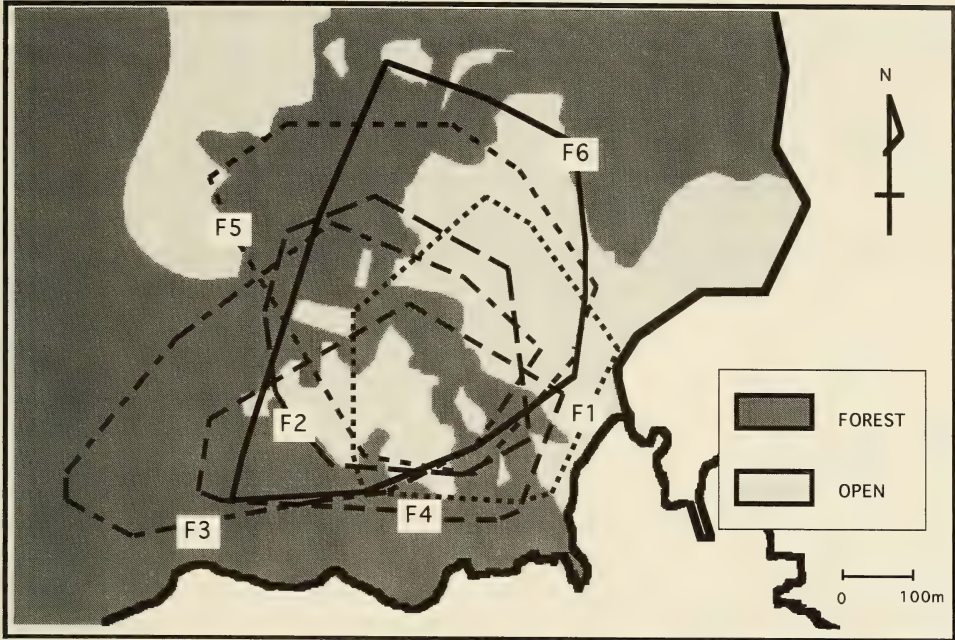Fig. 1. Home ranges of six female sika deer (F1–F6).

Fig. 2. Annual home rages of six female sika deer (F1-F6) in relation to hatbitat type.

2. Dynamic Interactions of Females

Annual home ranges were found to overlap considerably with each other (Fig. 2), suggesting that females permit each other to enter their own ranges, and that they form home range groups (Miura 1976). To evaluate the dependency in the simultaneous movements of pairs of females, we analyzed dynamic interactions among them (Table 3). Paired separations of less than 20 m were less frequent than those of more than 20 m for all dyads. Expected and observed number of paired separations <20 m were not significantly different except in one case (Table 3). In this case, only 4 out of 114 (3.5%) observed sepapations were < 20 m, thus evidence of dynamic interactions was limited to just two specific females. Generally, however, there was no dependency in the simultaneous movements of pairs of females, even though their home ranges overlapped.

Table 2. Degrees of range overlaps (*RO*) of female sika deer between seasons. *RO* was calculated as : size of overlap between two months (ha) / home range size (ha). Numbers of females are shown in parenthesis.

Jun.--Aug.	Jun.--Oct.	Jun.--Dec.	Jun.--Oct.	Aug.--Oct.	Oct.--Dec.
Jun.	Jun.	Jun.	Oct.	Oct.	Oct.
0.66±0.19 (N=6)	0.63±0.11 (N=6)	0.51±0.11 (N=5)	0.72±0.19 (N=6)	0.64±0.07 (N=6)	0.61±0.13 (N=5)
Jun.--Aug.	Aug.--Oct.	Aug.--Dec.	Jun.--Dec.	Aug.--Dec.	Oct.--Dec.
Aug.	Aug.	Aug.	Dec.	Dec.	Dec.
0.73±0.14 (N=6)	0.66±0.16 (N=6)	0.60±0.15 (N=5)	0.51±0.13 (N=5)	0.54±0.14 (N=5)	0.59±0.27 (N=5)

Tabl 3.   Frequencies of N paired and N^2-N unpaired distances, and those below and over the critical distance of 20 m.   *n.s.*: non-significant.

		F2		F3		F4		F5		F6	
		<20m	20m≦	<20m	20m≦	<20m	20m≦	<20m	20m≦	<20m	20m≦
F1	Paired	4	116	4	112	8	120	6	100	5	99
	Unpaired	452	13828	356	12984	586	15670	324	10806	391	10321
		$\chi^2=0$, *n.s.*		$\chi^2=.053$, *n.s.*		$\chi_2=1.84$, *n.s.*		$\chi_2=1.90$, *n.s.*		$\chi^2=.132$, *n.s.*	
F2	Paired			1	132	3	141	6	121	2	123
	Unpaired			309	17247	300	20292	332	15670	301	15199
				$\chi^2=.30$, *n.s.*		$\chi^2=.076$, *n.s.*		$\chi^2=3.12$, *n.s.*		$\chi^2=0$, *n.s.*	
F3	Paired					9	132	4	110	3	109
	Unpaired					668	19072	116	12766	147	12285
						$\chi^2=2.97$, *n.s.*		$\chi^2=5.794$, p<0.05		$\chi^2=1.027$, *n.s.*	
F4	Paired							2	116	2	122
	Unpaired							147	13659	158	15094
								$\chi^2=.045$, *n.s.*		$\chi^2=.035$, *n.s.*	
F5	Paired									7	114
	Unpaired									396	14124
										$\chi^2=3.127$, *n.s.*	

3. Habitat Preference

Home ranges of female deer included both forest and open habitats.   The seasonal changes of habitat preference for forest and open habitats were expressed by the electivity index (E_i)(Fig. 3). E_i for open habitats were positive
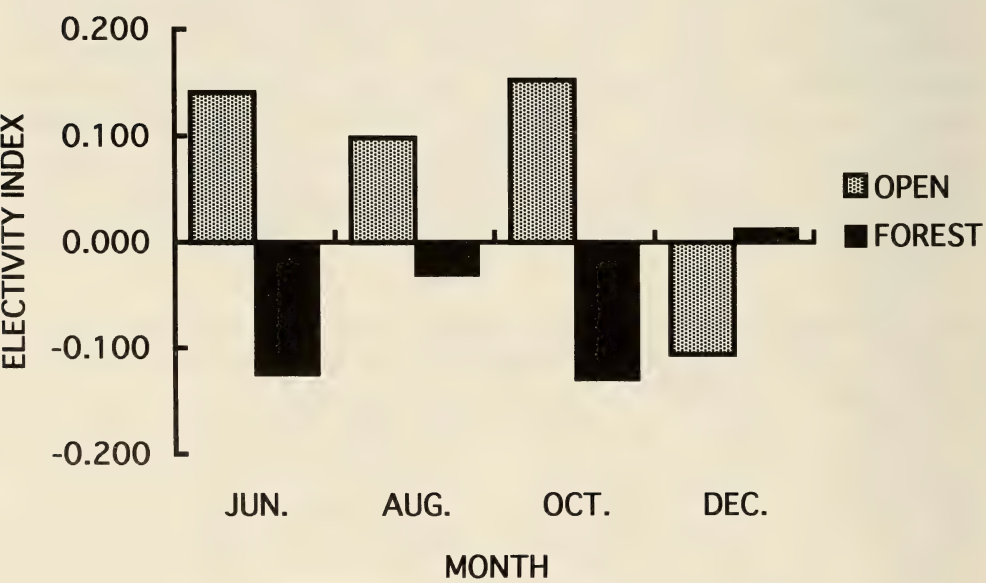
Fig. 3.   The seasonal changes in habitat preferences.

from June to October, but significantly negative in December (Friedman's test : $\chi^2 = 10.680$, $p < 0.05$). In contrast, E_i for forests was negative from June to October and positive in December, though they were not significantly different (Friedman's test : $\chi^2 = 7.800$, $p > 0.05$). E_i for open habitat was significantly positive in June (Student's t-test, $t = 3.069$, $p < 0.05$), while it was significantly negative in October ($t = 3.088$, $p < 0.05$). They were not significant in August ($t = 2.248$, $p = 0.0745$) and December ($t = -1.895$, $p > 0.05$). These results indicate that the females preferred open habitats from June to October and forest habitats in December.

DISCUSSION

Previous studies on the home ranges of sika deer have been made mainly on populations in the northern and central parts of Japan (Miura 1977, Maruyama 1981, Shigematsu *et al*. 1994, Yabe 1994). This is the first study from southern Japan. The mean size of the annual home ranges of resident females in the Hokkaido population was 325.2 ha (Yabe 1994). In Nikko in central Japan, the monthly home range sizes of males and females varied from 21.0 to 284 ha (Maruyama 1981), and in Chiba Prefecture, the female annual home ranges varied from 46.1 to 246.3 ha (Shigematsu *et al*. 1994). For the Nara population, the mean summer home range was 11.7 ha (Miura 1977). Compared to these results, the home ranges of the Nozaki population were considerably smaller. This difference may result from four factors.

First, in the northern areas seasonal migration serves to enlarge home range size, as leaves fall in autumn reducing cover, and as snow cover reduces food availability in winter, deer are forced to move to lower altitudes (Maruyama *et al*. 1976, Maruyama 1981, Ito and Takatsuki 1987, Takatsuki 1992). On Nozaki Island, in contrast, warm temperature, lack of snow, and the presence of evergreen forests enable the deer to remain in one area all year without migrating.

Second, home range size is related to body size. In Hokkaido, adult females weighed about 75.0 kg (Kaji *et al*. 1988, Yabe 1994), on Mt. Goyo (Takatsuki 1992) and in Chiba about 45.0 kg (Shigematsu *et al*. 1994), whereas the mean body weight of females on Nozaki Island was considerably less at just 32.2 ± 1.6 (*SD*) kg (N = 6). Even when the effect of migration was excluded, home range sizes varied among resident populations. Therefore, the small range size of females on Nozaki Island are a reflection of their smaller body weight.

Third, the type of vegetation affects home range size. For resident populations of sika deer, two types of home ranges (small stable type, and large) were reported in Chiba (Shigematsu *et al*. 1994) and in the Ashio population (Koganezawa and Satake, pers. comm.). The small stable type included *Zoysia*-type grasslands whereas the large type did not. Shigematsu *et al*. (1994) explained this variation by the presence of the highly productive *Zoysia*-type grasslands enabling the deer to thrive in smaller home ranges. Miyazaki

et al. (1977) suggested that highly productive *Zoysia*-type grasslands were also an important resource for the Nara deer population. On Nozaki Island, female home ranges also included *Zoysia*-type grasslands. The electivity index showed their high preference for open habitats in all seasons except winter (Fig. 3). It seems likely that the high productivity of *Zoysia*-type grassland facilitates the use of smaller home ranges by females in the Nozaki population.

Finally, deer density reached as high as 3.1/ha (Doi and Endo 1992) on Nozaki Island, which is higher than in other populations. Other studies of natural populations have reported highest densities as just 0.3/ha in Chiba (Ochiai and Asada 1993), 0.5/ha on Nakanoshima (Kaji *et al.* 1988), 0.6/ha on Kinkazan Island (Ito 1987) and 2.0/ha on Mt. Goyo (Takatsuki 1992). In addition to these four factors, spatial restriction on the island may also be an important factor affecting home range size.

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Distribution of cardiac musculature in the pulmonary venous wall of three species of the genus *Mustela*

Hideki Endo, Eiichi Hondo[1], Daishiro Yamagiwa[2], Teruhiko Wakayama[2], Masamichi Kurohmaru[2] and Yoshihiro Hayashi[2]

Department of Zoology, National Science Museum, Tokyo 169, Japan ; [1]Department of Veterinary Anatomy, Faculty of Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080, Japan ; [2]Department of Veterinary Anatomy, Faculty of Agriculture, The University of Tokyo, Tokyo 113, Japan
Fax. 03-3364-7104

Abstract. An examination was made of the distribution of cardiac musculature in the pulmonary venous wall of three *Mustela* species (ermine, American mink and ferret) of different body size. Only the ermine possessed cardiac myocytes in the tunica media of the intrapulmonary venous walls continuing from the left atrium, whereas the two other species had the musculature restricted to the large extrapulmonary vein. The distribution of the musculature is thought to depend on the body weight and heart rate of various species. These findings confirm the supposition that, whereas smaller mammals have more extensive cardiac musculature, even in the intrapulmonary venous wall, in order to regulate venous blood return and to resist reflux resulting from frequent atrial systole, the larger species may not require cardiac musculature in the distal vein.

Key words : cardiac myocyte, *Mustela*, pulmonary vein.

Arnstein (1877) and Stieda (1877) reported that striated musculature occurs in the intrapulmonary venous wall of several mammals. Since the late 1870s, it has been accepted that cardiac myocytes are present not only in the heart, but also in the pulmonary venous wall, in various mammals (Favaro 1910, Granel 1921, Karrer 1959a,b, Policard *et al.* 1959, Klavins 1963, Kramer and Marks 1965, Ludatscher 1968, De Almieda *et al.* 1975, Endo *et al.* 1992a,b,1995). Kramer and Marks (1965) suggested that the distribution of cardiac musculature differed between rodent species. This was confirmed by Endo *et al.* (1992a), who found that among three species of South American rodents differing in body size. The larger species, with lower heart rates, had cardiac musculature restricted just to the large pulmonary veins. So far however, the distribution of this musculature has not been compared among taxonomically closely-related species which would serve to avoid the phylogenetic influence.

The group *Mustela* (Carnivora) provides an eminently suitable opportunity for a study of comparative morphology, as there are various species differing in body size and heart rate within a single genus. In this study, we examined

the ermine (*Mustela erminea*) which has a body weight of 42-258 g and a heart rate of 300-420 beats/min. ; the American mink (*Mustela vison*) weighing 681-2310 g and a heart rate of 216-242 beats/min., and the ferret (*Mustela putorius*) weighing 500-1500 g and a heart rate of 272-414 beats/min. (Altman and Dittmer 1974, Walker 1983).   The aims of this study were to clarify the relationship between the distribution of cardiac musculature in the pulmonary vein in relation to body size and heart rate.

MATERIALS AND METHODS

One ermine (*Mustela erminea*), two American minks (*M. vison*) and one ferret (*M. putorius*) were examined in this study (Table 1).   Because the ermine is very rare, we examined a formalin-fixed specimen, considered to be adult on the basis of its head and body length.   No gross lesions were recognized in the heart or lung of animals during macroscopic pathological observation.

Table 1.   Biological data of the animals used in this study.

Species	Sex	Body weight (g)	Age (month)	Head and body length (mm)	Origin
ermine*	male	—	—	174	Minami-Aizu, Fukushima pre.
American mink	male	2250	13	435	maintained
	famale	1100	13	392	(conventional)
ferret	male	2080	9	420	maintained (conventiaonal)

*A formalin-fixed specimen from the National Science Museum (Specimen No.: M16000) : the body weight was not recorded and the age has not been determined.

The American minks and ferret were euthanized under deep sodium pentobarbital anesthesia.   They were perfused with physiological saline.   The lung lobes were excised with part of the left atrium, cut into small pieces, immersed in Bouin's fixative for 2-48 hr and dehydrated in ethanol.   The fixed tissues of ermine were immediately dehydrated.   The blocks were embedded in paraffin and cut into serial sections at 4 μm.   The sections were stained with phosphotungstic acid hematoxylin (PTAH) and/or Heidenhain's iron hematoxylin, and observed under a light microscope.

RESULTS

Cardiac musculature, with its characteristic blue or black PTAH and Heidenhain's iron hematoxylin stained cytoplasm with its thin layer structure

peculiar to cardiac myocytes, was easily recognized. In the ermine, cardiac musculature was confirmed to occur in the tunica media of both extra- and intra-pulmonary venous walls (Figs. 1-2). A few well-developed circular layers were found in the tunica media. The musculature was observed even in the primary branch of the intrapulmonary vein of about 300 μm in diameter (Fig. 2). In the American mink, cardiac musculature was found in the large extrapulmonary vein (Fig. 3). The musculature consisted of some longitudinal layers between tunica interna and thin fibrous tunica adventitia. However, the layers in the extrapulmonary area gradually diminished in number and disappeared completely in a portion of more than 1 mm in diameter (Fig. 4). In the ferret, the end of the musculature distribution was seen in the extrapulmonary venous wall (Fig. 5). The venous tunica media composed of well-developed collagen fibers was thicker than that in the American mink. In the intrapulmonary vein, collagen fibers were observed in the tunica media (Fig. 6), whereas cardiac myocytes were not discerned.

DISCUSSION

It is suggested that the pulmonary venous musculature may act as a pump and valve regulating the venous blood return and resisting reflux from the left
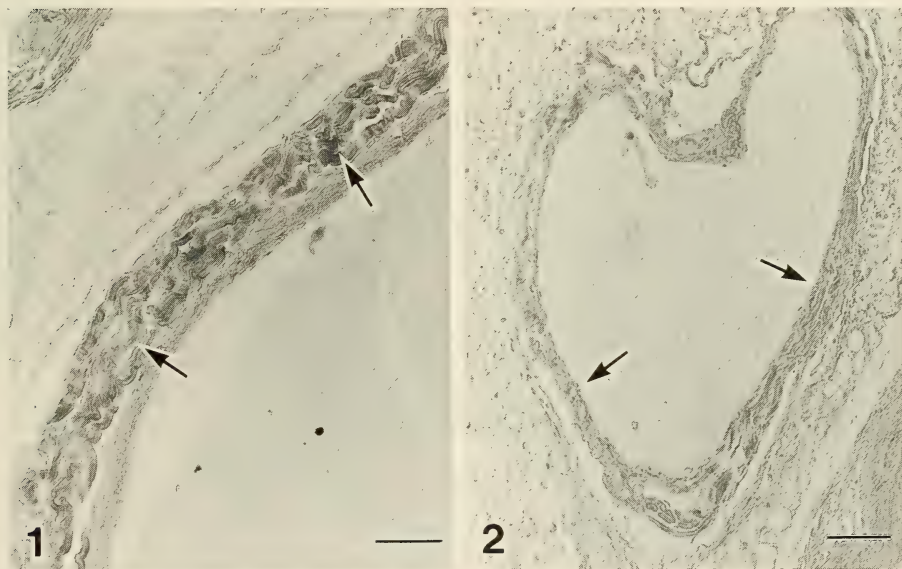
Fig. 1. Large extrapulmonary vein of the ermine. Some circular myocyte layers are observed in the tunica media (arrows); stained with phosphotungstic acid hematoxylin. Bar : 50 μm.

Fig. 2. Cardiac musculature is seen in the tunica media of intrapulmonary venous walls of the ermine (arrows). The portion is equivalent to the first branching point in the intrapulmonary vein; stained with phosphotungstic acid hematoxylin. Bar : 50 μm.
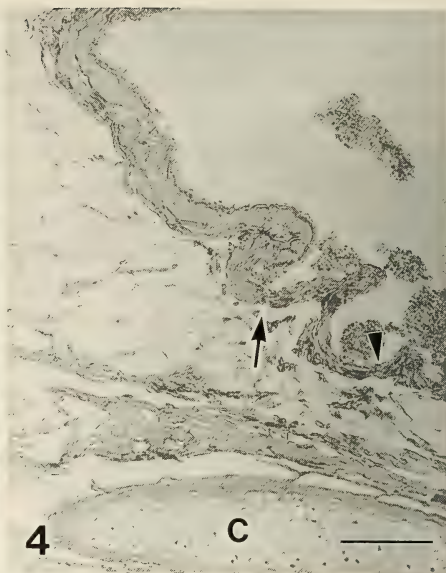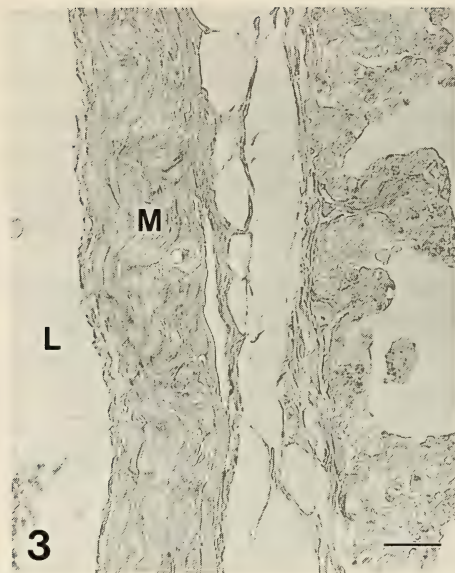
Fig. 3.   Longitudinal section of the extrapulmonary vein in the American mink.   Longitudinal cardiac musculature (M) is well-developed in the tunica media.   L, lumen ; stained with phosphotungstic acid hematoxylin.   Bar : 30 μm.

Fig. 4.   Extrapulmonary vein at hilus area of the American mink.   A few cardiac myocyte layers disappear in this portion (arrow).   Collagen fibers are shown in more distal venous wall (arrowhead).   C, tracheal cartilage ; stained with phosphotungstic acid hematoxylin. Bar : 100 μm.
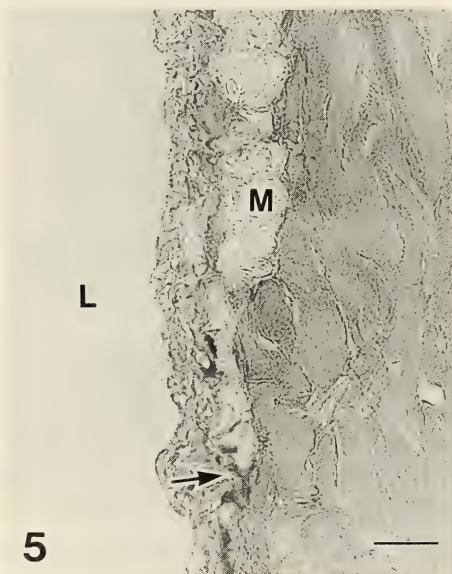

Fig. 5.   Longitudinal section of the extrapulmonary vein in the ferret.   The end of the cardiac musculature (M) is shown in the well-developed fibrous tunica media (arrow).   L, lumen ; stained with phosphotungstic acid hematoxylin.   Bar : 20 μm.

Fig. 6.   Small intrapulmonary vein of the ferret.   The tunica media is composed of collagen fibers (arrows) ; stained with phosphotungstic acid hematoxylin.   Bar : 50 μm.

atrium by active contraction (Kramer and Marks 1965, Endo *et al.* 1992a). From data pertaining to rodents, it seems likely that the distribution of the musculature is dependent on animal body size (Kramer and Marks 1965). Small rodents, with higher heart rates, may require a large area of musculature to assist venous blood return from the pulmonary circulation to the heart. Mammals with body weights of less than 500 g, such as the chinchilla (*Chinchilla laniger*), have extensive musculature in the intrapulmonary venous walls (Endo *et al.* 1992a). In order to demonstrate the musculature area-body size relationship, a study of closely-related species was necessary.

The comparative morphology of three *Mustela* species also demonstrates that smaller mammals, with body weights of less than 500 g, have musculature in the intrapulmonary venous wall, whereas the American mink and the ferret do not, as was indicated in our previous study on larger caviomorphs (Endo *et al.* 1992a). It is suggested that the extensive venous wall musculature in smaller mammals serves to avoid blood reflux caused by frequent atrial systole. The relationship between musculature distribution and body weight is likely also to be confirmed in groups other than carnivores and rodents in the future.

Only one or two animals were used in three species in this study. However, individual variation and sexual dimorphism have not previously been found in the musculature distribution of mammalian species (Kramer and Marks 1965, Ludatscher 1968, Endo *et al.* 1992a, b, 1995). Because we excised tissues from normal adult animals in this study, our histological findings indicate musculature distribution typical of each species. In contrast, the Siberian weasel *Mustela sibirica* differs significantly in body size and weight between males (650-820 g) and females (less than 500 g) (Walker 1983), making it an interesting species for a future study of sexual variation in musculature distribution.

Previous histochemical and biochemical studies have confirmed the presence of atrial natriuretic polypeptide (ANP) in the mammalian pulmonary vein and have suggested that the musculature has developed as an endocrine organ secreting ANP (Asai *et al.* 1987, Larsen *et al.* 1987, Endo *et al.* 1995). It will be interesting to examine whether the pulmonary venous wall in *Mustela* species may also be an ANP synthesis, storage and secretion organ.

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Preliminary study on kinematic gait analysis in mammals

Norihisa INUZUKA

Department of Anatomy, University of Tokyo, Faculty of Medicine, Hongo, Tokyo 113, Japan
Fax. 03-5800-6848

Abstract. The gait of several extant mammals was analyzed so as to provide basic data for the restoration of the terrestrial locomotion of extinct animals. An attempt has been made to establish the correlation between the gaits and the morphological data, as the latter can be obtained even from fossils. Animals walking naturally were recorded on videotape, appropriate frames were then printed for analysis. Five kinds of gaits are illustrated here with supporting graphs. In addition, some diagrams were drawn using variables of the gait cycle, the rhythm of limb work, the rhythm of locomotion and the hindlimb length ratio to the trunk. Changes in the four joint angles during a gait cycle were measured and graphed for comparison with each limb joint among mammals with four typical foot postures. The kind of gait was determined in relation to the limb length ratio, the gait cycle and the position of the center of gravity. The joint angle of limbs is in relation to foot posture. The wrist joint in walking is analogous to the knee joint in the degree, direction and timing of flexion.

Key words. gait analysis, joint angle, locomotion, mammal, restoration.

Some previous studies have been made on mammalian gaits (Sukhanov 1974, Gambaryan 1974, Hildebrand 1976), but not with the purpose of the restoration of the locomotion of extinct animals. There are two possible approaches to the restoration of the terrestrial locomotion of extinct mammals: firstly, to collect and analyze gait data from as many extant mammals as possible, so as to establish correlations between gait and the information obtainable from fossils such as body size, limb proportion and so on. Locomotion speed has not been addressed in this study, because it is difficult to estimate it exactly in extinct species. Secondly, the skeleton of an extinct animal can be mounted so that its limb joints can be moved, so as to confirm whether the assumed limb movements can actually be realized by the skeletal model.

A lateral-type limb posture for the desmostylian form which possesses mammalian joint morphological characteristics has been proposed (Inuzuka 1984). Notwithstanding its joint characteristics, if the stylopodium has the reptilian lateral-type limb posture, then the desmostylia should walk in a manner distinct from either reptiles or ordinary mammals.

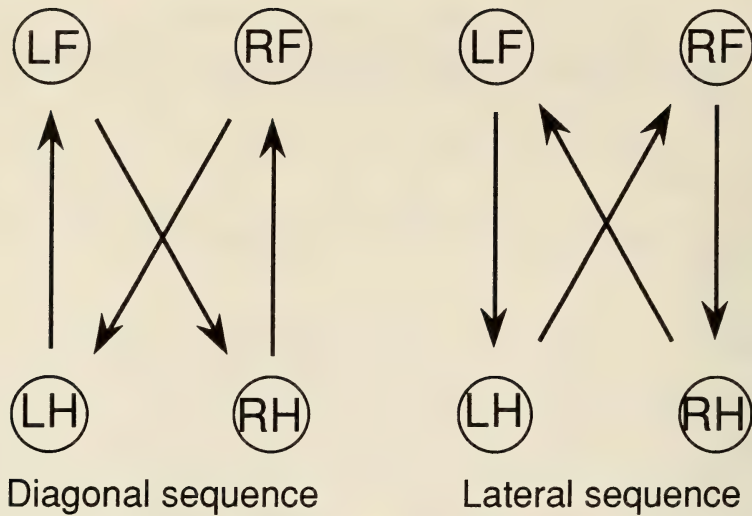In order to elucidate this situation an attempt was made to analyze the gait

Fig. 1.   Sequences of leg movements.

of living mammals first.   The restoration of locomotion could prove whether the form of a restored skeleton, considered using static methods, is reasonable or not.   The frozen moment of an actual step would be best selected as a display pose in exhibition.

In this study walking mammals were videotaped, and frames at 1/60 second intervals were analyzed.   Six kinds of gait were observed, and correlations between the gait cycle and the gaits, and between the foot posture and the flexion angles of limb joints in walking, became clear.

Terms used here relating to locomotion follow Gambaryan (1974), who divided terrestrial quadrupedal locomotion into symmetrical and asymmetrical gaits.   Symmetrical gaits are further divided into diagonal, or lateral, sequences depending on the order of footfall (Fig. 1).

Table 1.   Specimens examined for the gait analysis.

Order	Family	Species	English name
Primates	Cercopithecidae	*Macaca fuscata*	Japanese macaque
Carnivora	Canidae	*Canis aureus*	golden jackal
	Ursidae	*Ursus arctos*	brown bear
		Thalarctos maritimus	Polar bear
		Helarctos malayanus	sun bear
	Ailuropodidae	*Ailurus fulgens*	lesser panda
	Felidae	*Acinonyx jubatus*	cheetah
Proboscidea	Elephantidae	*Loxodonta africana*	African elephant
		Elephas maximus	Asiatic elephant
Perissodactyla	Equidae	*Equus caballus*	horse
		Equus ferus	Przewalski's wild horse
		Equus grevyi	Grevy's zebra
Artiodactyla	Hippopotamidae	*Choeropsis liberiensis*	pigmy hippopotamus
	Giraffidae	*Giraffa camelopardalis*	giraffe

Materials and Methods

Fourteen extant mammal species, representing nine families, and five orders, were videotaped at Ueno Zoo, Tama Zoo, and the Avalon Horse Riding School in Tokyo, Dusit Zoo in Bangkok and the Taklahn "Villege of Elephants" in Thailand (Table 1).

Animals walking naturally in a cage or a field were recorded on 8mm videotape using a telescopic lens held perpendicular to the ambulatory path, and as level with the animal as possible.   A full gait cycle in one direction was selected and edited from among the several series taken.   By means of a freeze frame video deck images every 1/60, 1/30 or 1/20 second of a cycle were displayed on a monitor, and successive frames were photographed with a motor-driven camera and printed.   These prints were used for the gait analyses.

One gait cycle is divided into the rise and fall of each foot, and the length of each phase was calculated from the number of pictures of the phase.   The support formula was derived from the change of the number of supporting limbs in a cycle, leading to the gait.   The support limb graph was made from the limb name and the supporting time of the limb provided, giving both the limb and locomotion rhythm.   For four representative mammals with typical foot postures, the sub-unguligrade Asiatic elephant, the unguligrade giraffe, the digitigrade cheetah and the plantigrade Polar bear, the flexion angles of all four limbs, the elbow, wrist, knee and ankle joints, were measured every 0.1 second so that representative line graphs could be drawn for comparison of each animal and each joint.   Because the position of a limb bone cannot be known exactly in life, it is represented by a line divided an angle into two equal parts between two lines representing the anterior and posterior margins of the leg.

Results

1. Gait and Support

Six kinds of gait were observed.   These consisted of five symmetrical gaits: very slow diagonal walk, slow trot-like walk, slow rack-like walk, normal walk and slow trot, and one asymmetrical gait: slow canter.   Among the symmetrical gaits, only the slow trot-like walk is a lateral sequence.   The slow trot is a trot, and the rest are all diagonal sequences.   These gaits, except for the slow trot, are illustrated in Figs. 2-6.   The number of each picture corresponds to that of its support graph.   The footfalls seen from above are shown with black circles in the middle of the support graphs, and so the upper circles denote the left side and the lower the right side.   Four bars are distributed in pairs above and below the footfall formulas, the outer bars corresponding to the hindlimbs and the inner ones to the forelimbs.   The crosshatched part of the bar denotes the support phase and the nonhatched part the free transit
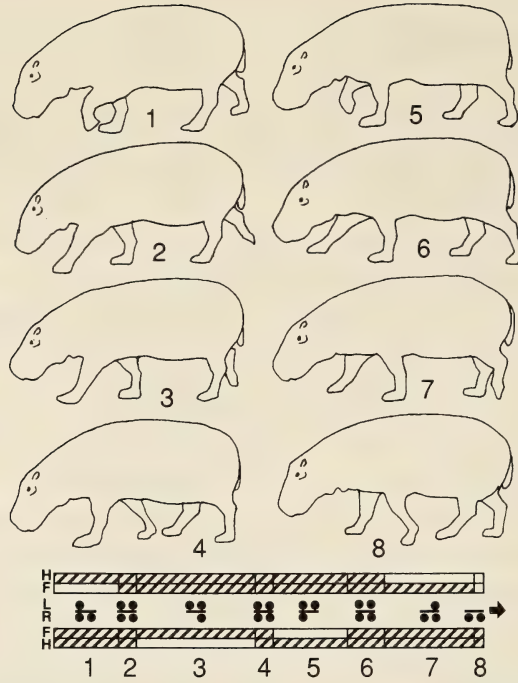
Fig. 2. Very slow diagonal walk of the pigmy hippopotamus and its support graph. For explanation, see in text.
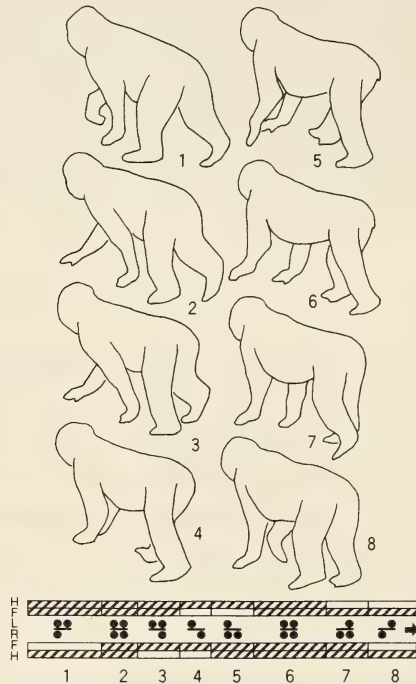

Fig. 3. Slow trot-like walk of the Japanese macaque and its support graph. For explanation, see in text.
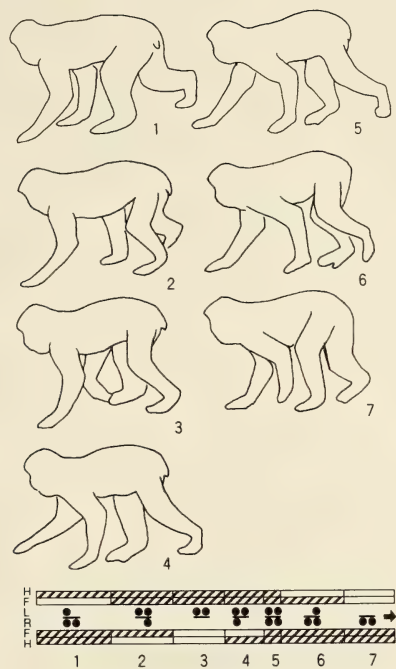
Fig. 4. Slow rack-like walk of the Japanese macaque on a downward slope and its support graph. For explanation, see in text.
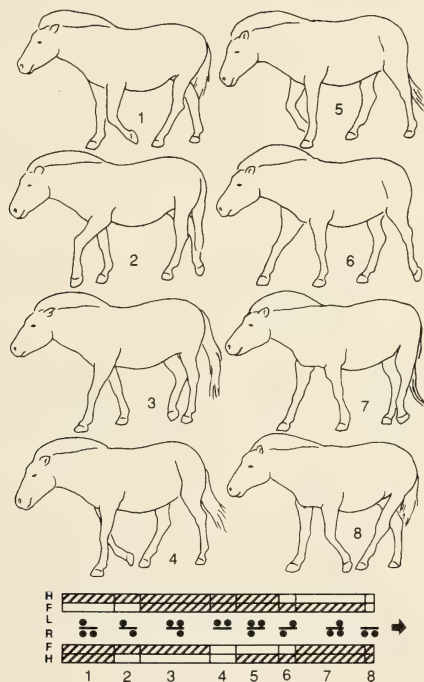

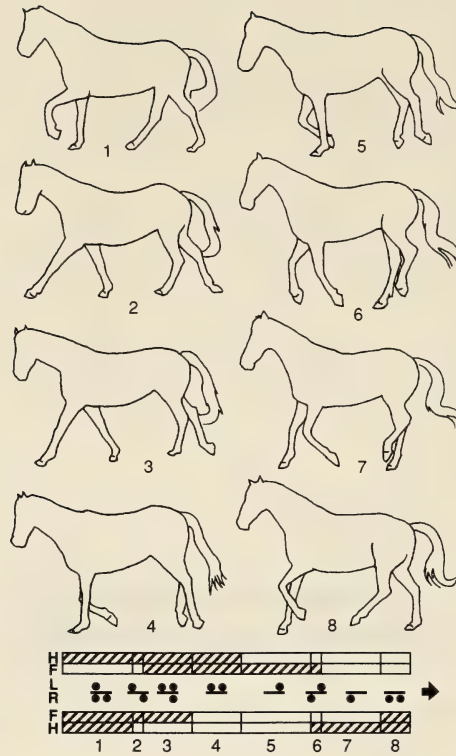Fig. 5. Normal walk of Przewalski's horse and its support graph. For explanation, see in text.

Fig. 6. Slow canter of the horse and its support graph. For explanation, see in text.

phase. The bar length is determined in proportion to the duration of each phase.

2. Gait Cycle and Limb Rhythm

Correlation between the gait cycle and the rhythm of limb work reveals that the latter decreases as the cycle becomes shorter, that is, the duration of limb support shortens, as the walking speed increases (Fig. 7). Correlation between the gait cycle and the hindlimb length ratio to the trunk length reveals that the gait cycle lengthens and the leg length, in proportion to the trunk, shortens, as body size increases in the elephant (Fig. 8) . In the carnivores, the cycle tends to be inversely related to the hindlimb length ratio. In the diagram (Fig. 9) of examining the correlation between the rhythm of limb work and the rhythm of locomotion, values of 0–5% on the vertical scale correspond to the rack or pace, 5–45% corresponds to the diagonal sequence, 45–55% to the trot, and more than 55% to the lateral sequence. In the diagonal sequence, Fig. 9 reveals that the slow rack-like walk changes to the normal walk as the speed increases.
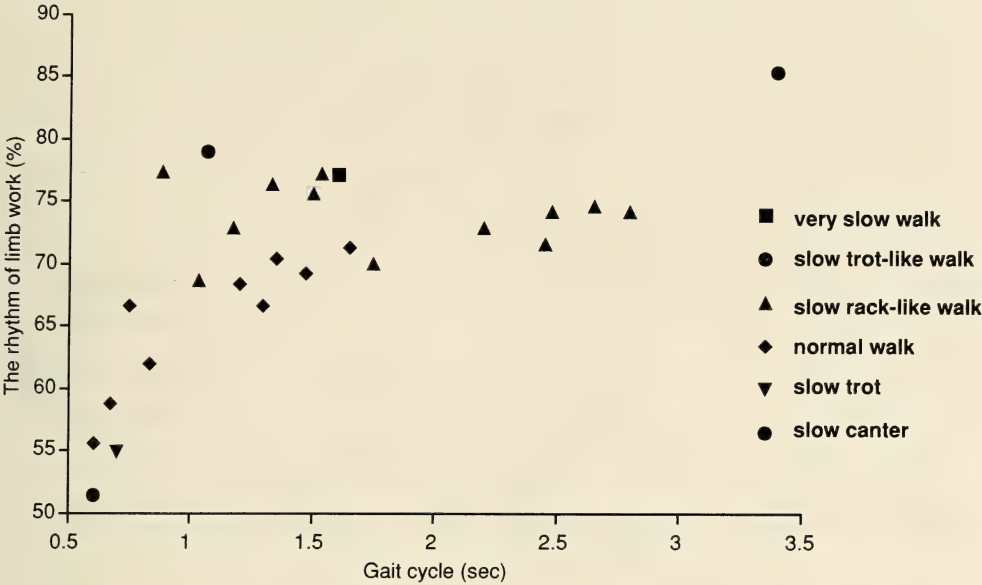
Fig. 7.   Scattergram showing correlation between the gait cycle and the rhythm of limb work.
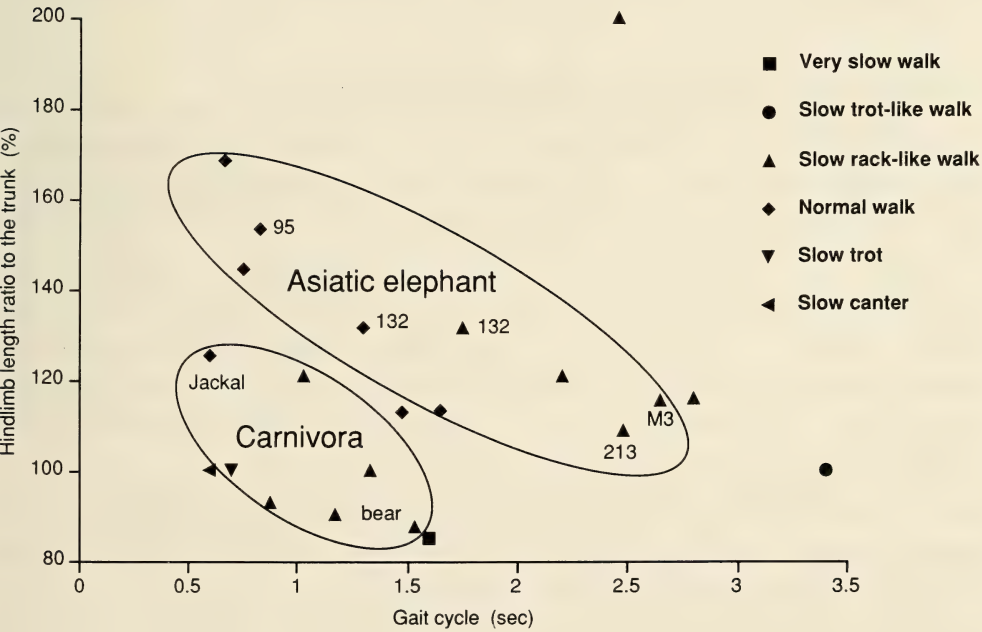

Fig. 8.   Scattergram showing correlation between the gait cycle and the hindlimb length ratio to the trunk.   Numerals show the shoulder heights in cm and M3 refers to age using the last molar.
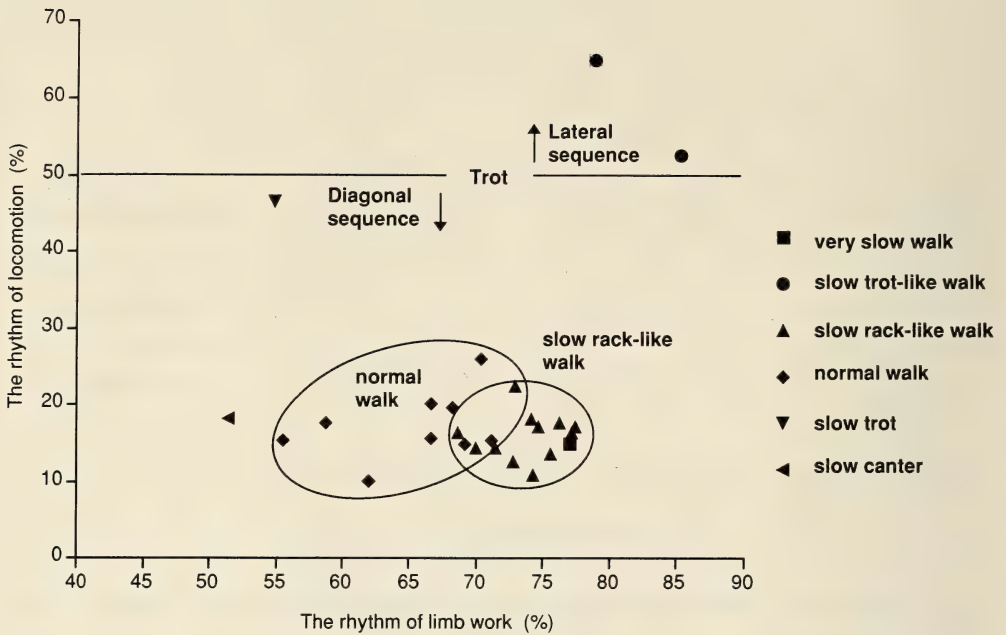
Fig. 9.   Scattergram showing correlation between the rhythm of limb work and the rhythm of locomotion.

3. A Comparison of Flexion Angles of Joints

In the sub-unguligrade Asiatic elephant, the elbow, knee and ankle joints generally maintain an extended position and only the wrist joint flexes markedly in the free transit phase.   The knee joint flexes more than the elbow joint in the free transit phase.   The ankle joint does not flex, even in the free transit phase, and varies little in angle (Fig. 10).

In the unguligrade giraffe, three joints are always flexed, except for the wrist joint dorsiflexing at an angle of 10° during the support phase.   The flexion angle of the knee joint tends to increase over time even during the support phase, suggesting that an unguligrade animal walks mainly using its knees rather than its hips, because the change of the angle is comparable to the angle between the legs distal to the knee joint (Fig. 11).

In the digitigrade cheetah, three joints are always flexed at angles of about 40°, except for the wrist joint which flexes dorsally at an angle of 20° during the support phase.   The ankle joint is always flexed more than the wrist and gradually extends during the support phase (Fig. 12).

In the plantigrade Polar bear, the wrist joint is flexed dorsally at 60° and the ankle at 90° during the support phase.   The knee joint reveals the maximum flexion just before foot-off, while the ankle joint plantarflexes once just before.
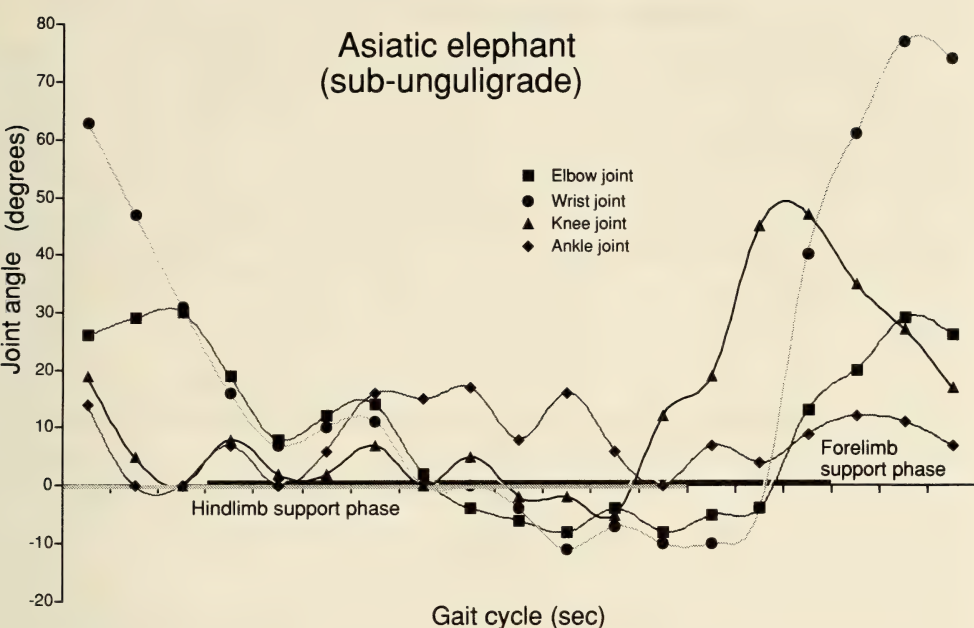
Fig. 10.   Joint angles of the sub-unguligrade Asiatic elephant.   Time, from the footfall of the hindlimb, is shown on the X-axis, and the flexion angle, from the position of extension, on the Y-axis.   In the ankle joint, dorsiflexion is indicated with + and plantarflexion with −.
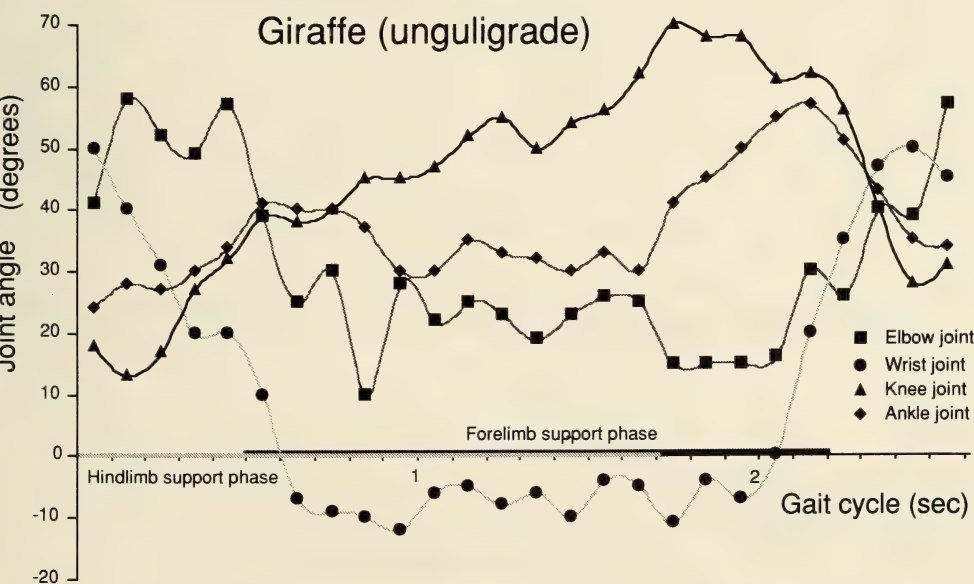

Fig. 11.   Joint angles of the unguligrade giraffe.   Time, from the footfall of the hindlimb, is shown on the X-axis, and the flexion angle, from the position of extension, on the Y-axis.   In the ankle joint, dorsiflexion is indicated with + and plantarflexion with −.
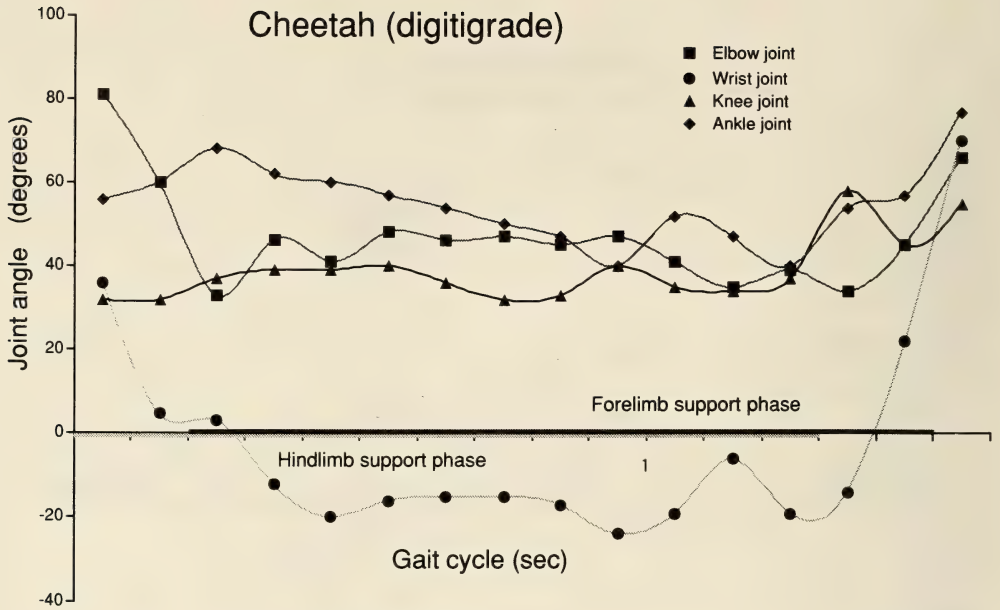
Fig. 12.   Joint angles of the digitigrade cheetah.   Time, from the footfall of the hindlimb, is shown on the X-axis, and the flexion angle, from the position of extension, on the Y-axis.   In the ankle joint, dorsiflexion is indicated with + and plantarflexion with−.
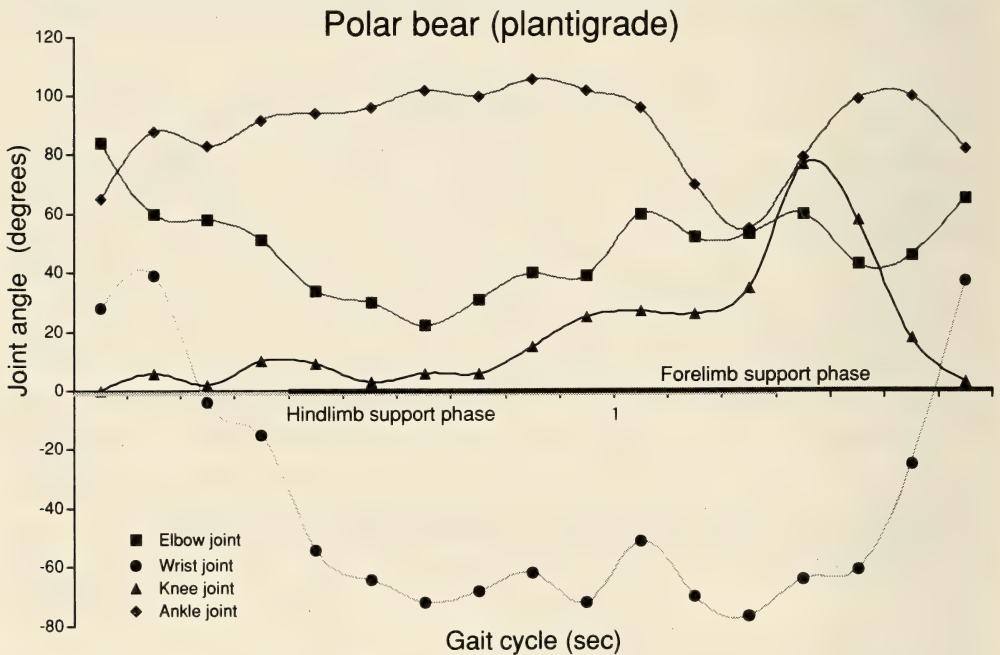

Fig. 13.   Joint angles of the plantigrade Polar bear.   Time, from the footfall of the hindlimb, is shown on the X-axis, and the flexion angle, from the position of extension, on the Y-axis. In the ankle joint, dorsiflexion is indicated with + and plantarflexion with−.

4. Comparison of Joint Flexion Angles

In all species examined, except for the Asiatic elephant, the elbow joint is flexed even during the support phase, and flexes even further during the free transit phase (Fig. 14). The wrist joint is mostly extended during the support phase, except in the plantigrade Polar bear where it is in dorsiflexion. It flexes further in the free transit phase in all species studied, with the greatest variation observed in the elephant and the bear. The change in angle, between the maximum and minimum, for the wrist, is the largest among the four joints (Fig. 15). During the support phase the knee joint is extended in the elephant and the bear, whereas it is flexed in the giraffe and the cheetah. It flexes further in the free transit phase in all species and this motion is most distinctive in the bear. The change in angle, between the maximum and minimum, for the knee, is the smallest among all joints (Fig. 16). The angle of the ankle joint varies little on the whole, though each species is clearly distinct as a result of differences in foot posture. The ankle flexes just before the foot is raised and this motion is most distinctive in the bear (Fig. 17).

Comparing the flexion angles of joints during the support phase between species, the orders for the elbow and ankle joints are the same, and as follows: sub-unguligrade < unguligrade < digitigrade < plantigrade. The order for the wrist joint is the exact reverse. The order for the knee is the same as the wrist except in the Asiatic elephant.
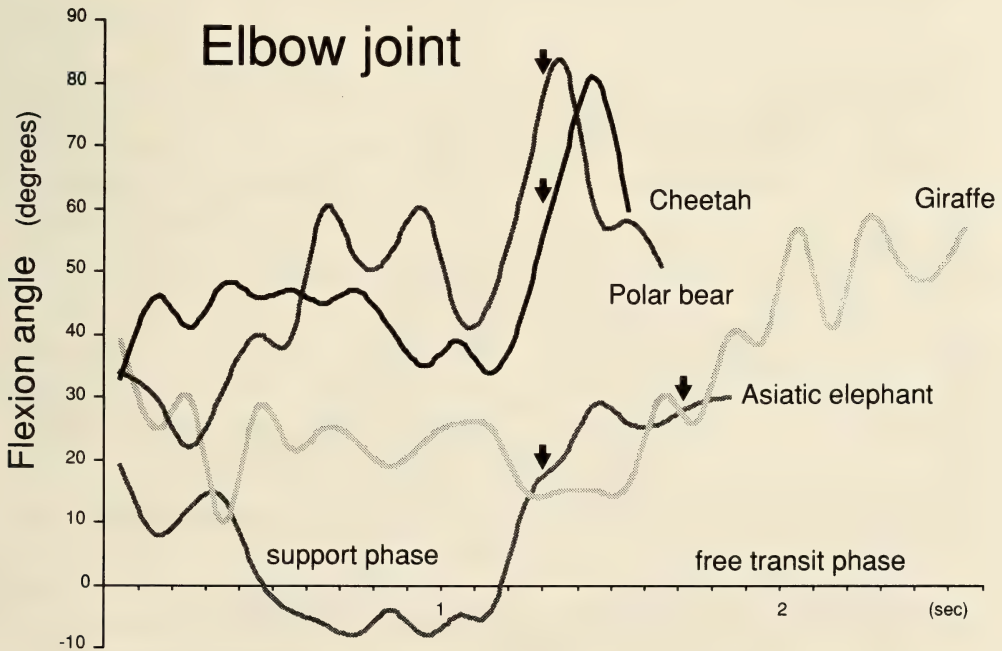

Fig. 14. Change of angles in the elbow joint. Time from the footfall of both limbs is shown on the X-axis, and the moment when the foot is raised, is shown by arrows.
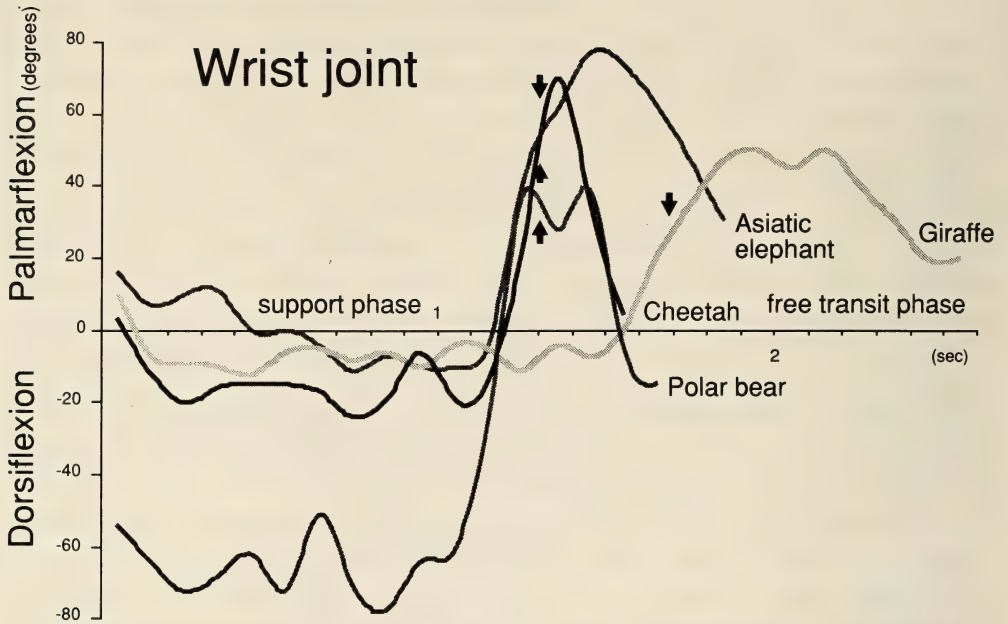
Fig. 15.   Change of angles in the wrist joint.   Time from the footfall of both limbs is shown on the X-axis, and the moment when the foot is raised, is shown by arrows.
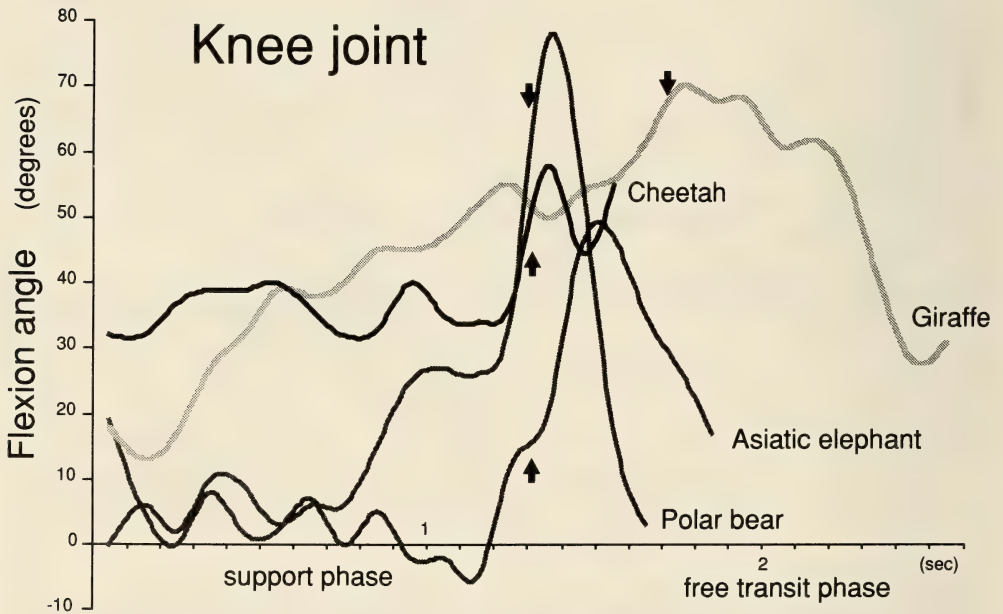

Fig. 16.   Change of angles in the knee joint.   Time from the footfall of both limbs is shown on the X-axis, and the moment when the foot is raised, is shown by arrows.
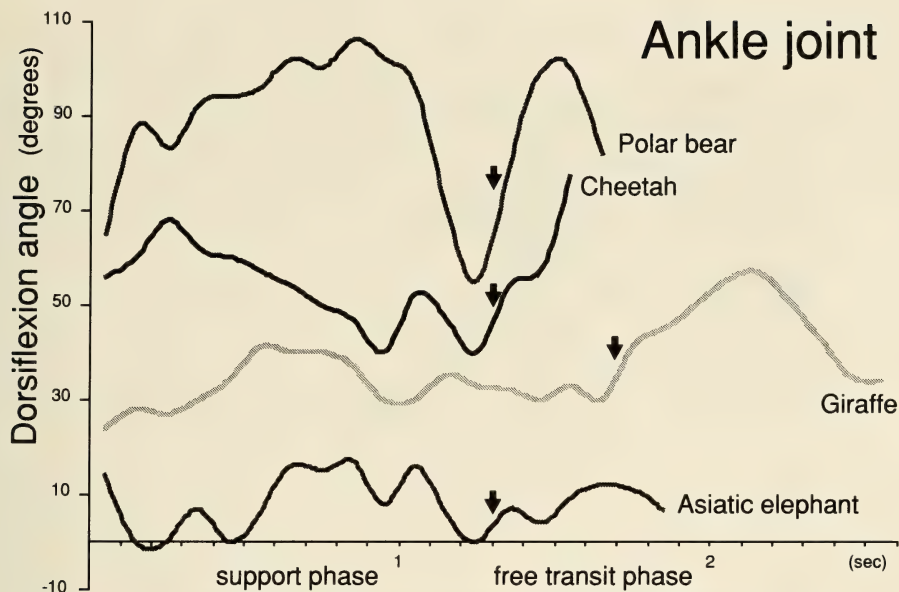
Fig. 17. Change of angles in the ankle joint. Time from the footfall of both limbs is shown on the X-axis, and the moment when the foot is raised, is shown by arrows.

Discussion

Usually, primates walk using the lateral sequence, making then different from other mammals. However, primates have been observed walking downhill in a diagonal sequence supporting Iwamoto and Tomita's (1966) theory that gait type is related to the center of gravity, because the forelimbs support more weight than usual on a downward slope.

A diagram of correlation between the rhythm of limb work and the rhythm of locomotion corresponds to a diagram (Fig. 18) by Hildebrand (1976).

Common features shared by the Asiatic elephant, giraffe, cheetah and Polar bear are that the wrist joints vary most markedly during a cycle, show marked flexion during the free transit phase, and also that the moment of maximum flexion is earlier in the wrist than in the elbow in the forelimb, and earlier in the knee than in the ankle in the hindlimb.

Comparison of flexion angles of joints during the support phase, indicates that: foot posture is closely related to the angles of limb joints; the elbow is analogous to the ankle and the wrist to the knee, and that the sub-unguligrade posture of the elephant is unique.

The other results from the comparison of joint angles may be summarized as follows: The knee joint is not so synchronized with the ankle as is the elbow with the wrist. The wrist joint is analogous to the knee joint in the degree, direction and timing of flexion. This is another analogy of fore- and hind-limbs differing from the analogy of the forearm and foot, which explains the
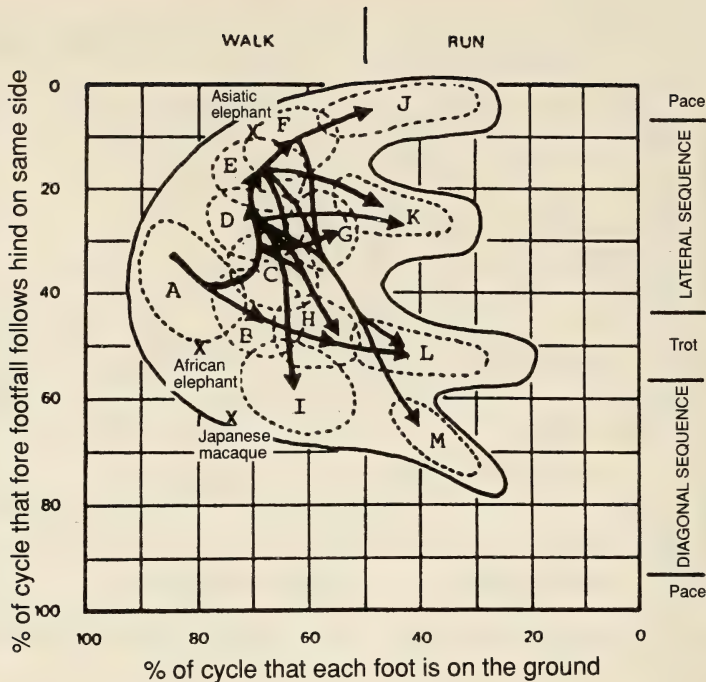
Fig. 18. A diagram showing the rhythm of limb work and the rhythm of locomotion after Hildebrand (1976).

position of the elbow and knee which appears with the emergence of the mammals.

Extrapolating from these results, one of the possible gaits of the desmostylian is a very slow diagonal walk using a diagonal sequence.   Estimation of locomotion velocity, maximum velocity and gait are subjects for future study.

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Short Communication

Spatial segregation between the Japanese field vole *Microtus montebelli* and the Japanese wood mouse *Apodemus speciosus* on the Naka River flood plain, northern Kanto

Kohtaro URAYAMA*

Department of Biology, Faculty of Science, Ibaraki University, Mito 310, Japan

The Japanese field vole *Microtus montebelli* and the Japanese wood mouse *Apodemus speciosus* often occur sympatrically in Honshu and Kyushu, Japan, whereas only *A. speciosus* occurs in Shikoku and Hokkaido. These two species differ in several ecological features. For example, *M. montebelli* inhabits grasslands, including flood plains, and eats mainly grass leaves, stems and roots, while *A. speciosus* occurs in a wide range of habitats from woodlands and secondary forests to grasslands, and eats nuts, berries, seedlings and insects (Tatsukawa and Murakami 1976). Despite these differences, the two species often occur together in cultivated fields (Kaneko 1973, 1979) and flood plains (Kaneko 1979, Saito *et al.* 1980, Sasaki *et al.* 1989). Kaneko (1979) investigated the habitat preferences of the two species using snap-traps in western Honshu, and suggested that *A. speciosus* was subordinate in habitats where *M. montebelli* predominated.

In this study the spatial distribution of *M. montebelli* and *A. speciosus* sympatrically inhabiting a flood plain with heterogeneous vegetations is examined and the interspecific interactions between the two rodent species are briefly discussed.

STUDY AREA AND METHODS

The field study was conducted along the Naka River flood plain, at Mito (36°25' N, 140°26' E), central Japan. The vegetation of the study area was heterogeneous dominated by the perennial reed *Phragmites communis* and the perennial forb *Solidago altissima* with sparse patches of shorter grasses and forbs (Fig. 1).

A total of 64 trapping stations, spaced at seven meter intervals, were set on the flood plain to form an approximately 0.25 ha (49 m × 49 m) open grid (Fig. 1). A single Sherman-type live-trap was placed at each trap station. Traps baited with sunflower seeds and were set at about 17 : 00 hrs and checked the

*Present address : Laboratory of Ethology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu 183, Japan
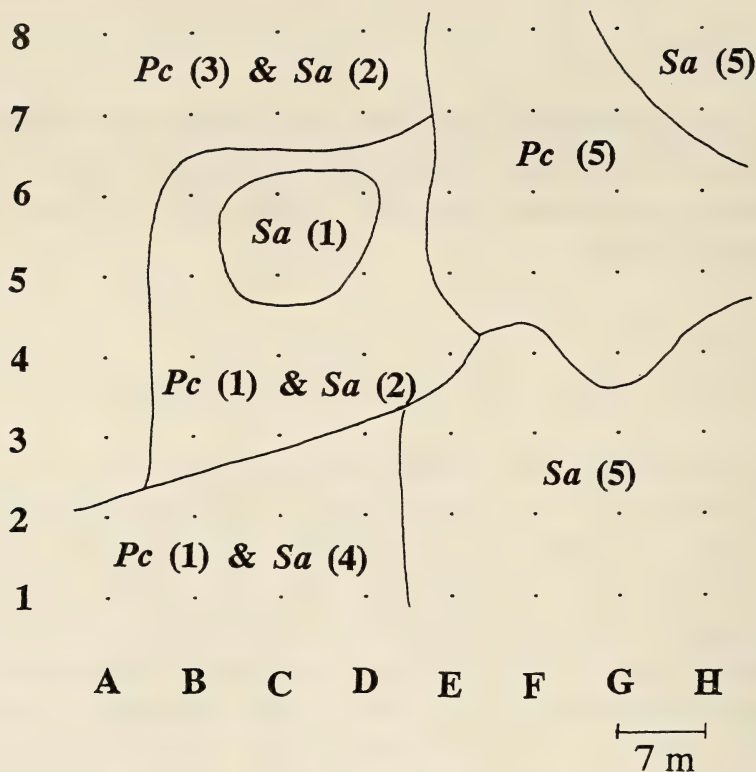
Fig. 1. Distribution and quantity of the two dominant plants, *Phragmites communis (Pc)* and *Solidago altissima (Sa)*, in the study area along the Naka River. Numerals in parentheses indicate the coverage class of these two species (0 : ≤1%, 1 : 1-10%, 2 : 10-25%, 3 : 25-50%, 4 : 50-75%, 5 : 75-100%).

following morning at about 08 : 00. Trapping took place 13 times during the period from 6 June to 18 July 1991. All individual *M. montebelli* and *A. speciosus* caught were sexed, weighed (to the nearest 0.5 g with a spring balance) and marked individually by toe-clipping. The trap location was also noted. Trappings were repeated every two or three days during the research period.

Microhabitat segregation between *M. montebelli* and *A. speciosus* was examined using multiple regression analysis based on the total number of captures at each trap station for *M. montebelli* (variable X_1) and for *A. speciosus* (variable X_2) and the coverage class (0 : ≤1%, 1 : 1-10%, 2 : 10-25%, 3 : 25-50%, 4 : 50-75%, 5 : 75-100%) of two dominant plants, *Solidago altissima* (variable X_3) and *Phragmites communis* (variable X_4). In the analysis for *M. montebelli* the criterion variable was X_1 and the explanatory variables were X_2, X_3 and X_4, and for *A. speciosus* the criterion variable was X_2 and the explanatory variables were X_1, X_3 and X_4.

RESULTS AND DISCUSSION

A total of 318 captures of 78 individuals were made during the study, of which 261 captures (82.1%) of 63 individuals were of *M. montebelli* and 56 captures (17.6%) of 14 individuals were of *A. speciosus*. The only other small mammal captured was a single (0.3%) Japanese white-toothed shrew (*Crocidura dsinezumi*). Population densities, estimated using the Jolly-Seber method, for both species from 6 June to 18 July showed little fluctuation, with mean densities of 126.2±8.1 (*SD*)/ha for *M. montebelli* and 24.2±5.7/ha for *A. speciosus*. Kanamori and Tanaka (1968) suggested that the typical population density of *M. montebelli* was 50 /ha, while the maximum density so far reported was 1120 /ha on the flood plain of the Tone River (Kitahara 1980). The density of the Naka River flood plain population is known to have been 171 /ha in the autumn of 1990 (Inada, pers. comm.). The population density of *A. speciosus* is generally fairly constant within a range of 10-50 /ha (*e.g.,* Murakami 1974, Doi and Iwamoto 1982). In the present study, the population density of *M. montebelli* was somewhat higher than the typical level, while that of *A. speciosus* was relatively low, indicating that *M. montebelli* was the predominant species in this area.

The mean lengths of home ranges (based on the minimum polygon method) of individuals caught more than four times during the research period were 16.1±5.4 (*SD*) m for *M. montebelli* and 26.6±8.8 m for *A. speciosus*. The facts that the mean range lengths for both *M. montebelli* and *A. speciosus* were longer than the distances between neighboring traps (7 m), and that the home ranges of most animals included several trap stations, suggest that multi-collisions of animals at each trap station was not so frequent as to greatly affect the observed number of captures.

Captures of *A. speciosus* were concentrated along southern and eastern edges of the grid, whereas *M. montebelli* was less frequently captured there than in other parts of the area (Fig. 2). The number of *M. montebelli* captured at each station was negatively correlated with that of *A. speciosus* ($r=0.496$, $n=64$, $p<0.001$). Multiple regression analysis showed that the most important variable determining the spatial distribution of *M. montebelli* was the presence of *S. altissima* (X_3), while the most important variable affecting *A. speciosus* was the distribution of *M. montebelli* (X_1)(Table 1). These results support Kaneko's (1979) conclusion that *A. speciosus* hardly intrudes into microhabitats where *M. montebelli* is predominant. The present results also support Kaneko's (1982) supposition that *M. montebelli* is the dominant rodent in Tohoku, Kanto and Chubu districts, while it is subordinate from Kansai to Kyushu districts.
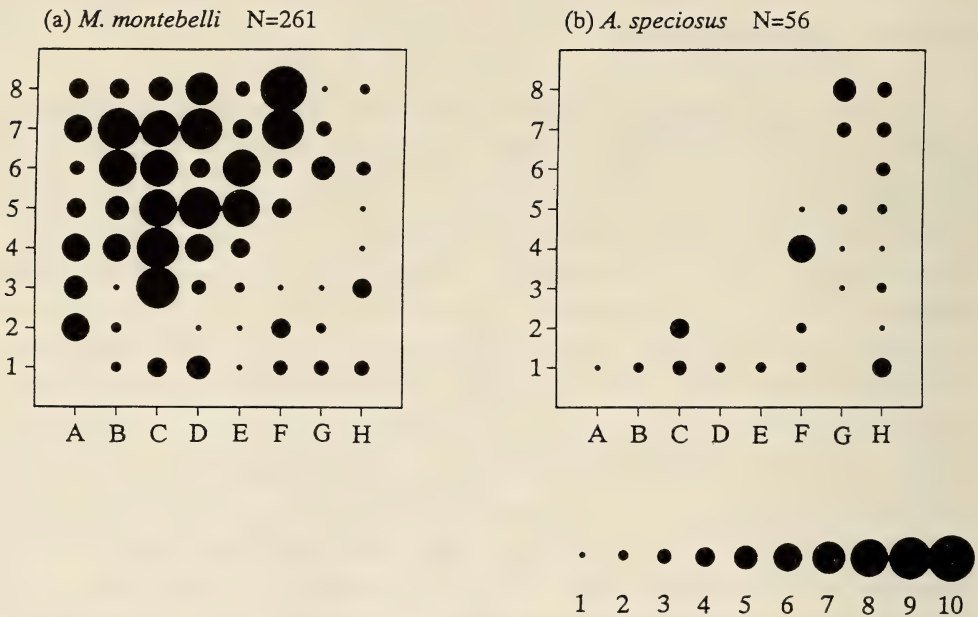
Fig. 2.   Total number of captures at each trap station for (a) *Microtus montebelli* and (b) *Apodemus speciosus* between 6 June and 18 July 1991. Circle sizes indicate the number of voles and mice captured.

Table 1.   Results of the multiple regression analysis on the spatial distribution of *Microtus montebelli* and *Apodemus speciosus* at each trap station of the study grid. Variables X_1, X_2, X_3 and X_4 are the number of *M. montebelli* and of *A. speciosus* captured and the degree of coverage of *Solidago altissima* and of *Phragmites communis*, respectively.

Criterion variable	Explanatory variable	Regression coefficient	Standerd error	*t*-value	d.f.	Probability
M. montebelli	X_2	−0.596	0.207	2.878	60	0.006
(X_1)	X_3	−1.085	0.314	3.453	60	0.001
	X_4	−0.569	0.296	1.922	60	0.059
A. speciosus	X_1	−0.204	0.071	2.878	60	0.006
(X_2)	X_3	0.229	0.199	1.150	60	0.255
	X_4	0.156	0.177	0.877	60	0.384

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Short Communication

Longevity of captive shrews in Hokkaido

Satoshi OHDACHI*

Institute of Low Temperature Science, Hokkaido University, Kita-ku, Sapporo 060, Japan
Fax. 011-706-7142, E-mail. ohdachi @ bio. hokudai. ac. jp

Information concerning the longevity of animals is invaluable for various biological studies. The longevity of some shrew species (Soricidae) has been reported both from the wild and in captivity (Churchfield 1990). Churchfield (1990) also showed that captive shrews tended to live longer than those in the wild in general, because of the preferential conditions in the laboratory. For a shrew species of Hokkaido, Inoue (1990) reported a maximum estimated life span of 511 days for *Sorex unguiculatus* in the field. For *S. unguiculatus* in captivity, Yokohata (1989) reported a maximum keeping period of 493 days and estimated that the oldest might live for 710–830 days. However, longevity for *Sorex caecutiens* and *S. gracillimus*, other common soricine species in Hokkaido, is little known. In the present study, all the three species were kept in the laboratory, slightly modifying Yokohata's (1989) rearing method. The purpose of the present study is to report the longevity of captive *Sorex caecutiens* and *S. gracillimus* along with that of *S. unguiculatus*.

MATERIALS AND METHODS

1. Animals examined

Shrews were collected in June 1992 and 1993 from Tomakomai (Yufutsu moor), and in June 1992 and August 1993 from Horonobe near the Teshio Experimental Forest of Hokkaido University. The methods of capturing shrews were essentially the same as those described by Ohdachi (1992). Seventeen *S. caecutiens*, 22 *S. gracillimus*, and 28 *S. unguiculatus* were used for analysis.

The animals examined for the present report were originally used for behavioral laboratory experiments (Ohdachi 1994, 1995a, b). During the experiments, laboratory conditions were maintained at either 16L8D, 20°C or 10L14D, 5-15°C. After the experiments, the animals were kept in order to record their longevity, but photoperiodic cycle was no longer controlled. Mixed paste diets of pork meat, pork liver, canned tuna, dog food and rabbit pellets were supplied every day. In addition to the mixed pastes, living mealworms (*Tenebrio* sp.), living earthworms and frozen silkworm pupae (*Bombyx mori*) were given occasionally. The supplementary natural foods seemed to contribute to the

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greater longevity of these captive shrews.   Animals that were sexually im-
mature when captured experienced neither copulation, pregnancy, nor parturi-
tion during their lives.   The most significant difference between Yokohata's
(1989) rearing methods and those in the present study was that I always kept
cages clean while Yokohata did not.   See Ohdachi (1994) for more detailed
methods.

2. Estimation of Longevity

Two methods for estimating longevity were used depending on the age of
the shrews when captured ; the first is for young of the year, and the second for
individuals that have overwintered.   Ages at capture were assessed on the
basis of wear to hair and body weight (Abe 1958, Ohdachi and Maekawa 1990).

The young shrews that were caught were all considered to be fully indepen-
dent from their mothers.   Inoue (1990), who reviewed the literature concerning
lactation periods (≈ the period from birth to independence) of six soricine
species (*Sorex cinereus, S. vagrans, S. araneus, Cryptotis parva, Neomys fodiens*
and *Blarina brevicauda*), concluded that the lactation periods ranged from 16 to
30 days.   Inoue (1990) also estimated that the lactation period of *S. un-
guiculatus* in the field averaged 27.6 days.   Churchfield (1990) considered that
the period from birth to complete independence in *S. araneus* lasted 25 days.   In
the light of these studies, it is assumed that for the three soricine species in
Hokkaido, the period from birth to independence averages 25 days.   Therefore,
in order to estimate the life span of the individuals that were captured as
youngsters, 25 days were added to their survival periods in the laboratory, thus,
giving a minimum estimate of longevity, as 25 days is the estimated minimum
age of the young animals captured from the wild.

Most soricine species bear young from spring to autumn (mostly in spring)
and new-born individuals, usually, do not become sexually mature until the
following spring (*e.g.*, Crowcroft 1957, Churchfield 1990).   In *S. unguiculatus* of
central Hokkaido, most females bear offspring between April and September
(Inoue 1990).   Pregnant female *S. caecutiens* and *S. gracillimus* were recorded
no later than in late September, although some females are known to survive
until November (Ohdachi unpublished data).   It is assumed, therefore, that the
last possible birth date of shrews in Hokkaido is October 1st.   Thus, in order
to estimate the age of shrews that were captured after they had overwintered
(*i.e.*, sexually mature individual), the period from October 1st of the previous
calendar year to the date of capture was added to the period survived in the
laboratory.   Again, this method of estimation provides only a minimum life
span, since the estimated period survived in the wild is also a minimum.

RESULTS AND DISCUSSION

Most wild-captured shrews were successfully introduced into the labora-
tory, although several died during transportation.   Two out of 17 *S. caecutiens*,
2 out of 22 *S. gracillimus*, and 4 out of 28 *S. unguiculatus* which were success-

fully introduced to the laboratory died within the first week. Most *S. caecutiens* and *S. unguiculatus* which survived the first week in the laboratory survived for more than 100 days. In contrast, 13 out of the 20 surviving *S. gracillimus* died within 100 days (mean = 52.4 days). In most cases, animals died suddenly without apparent symptoms, and the cause of death were unknown.

The maximum estimated life span for *S. caecutiens* was 609 days, for *S. gracillimus* 419 days and for *S. unguiculatus* 946 days (Fig. 1). The maximum life span for *S. caecutiens* would, in fact, have been longer, had it not died as a result of its water supply failing. The maximum of 946 days for *S. unguiculatus* reported here is one of the longest life span records among the Soricinae (Churchfield 1990).

Churchfield (1990) pointed out that larger shrew species tended to live longer than smaller species, which seems to be related to activity and basal metabolic rates. *S. unguiculatus* is the largest species and *S. gracillimus* the smallest among the three species in Hokkaido, and in the present study *S. gracillimus* tended to live shorter lives than the other two species (Fig. 1). It seems, therefore, that interspecific differences in the maximum estimated life spans of Hokkaido shrews seems to be related to body size, as pointed out by Churchfield (1990).
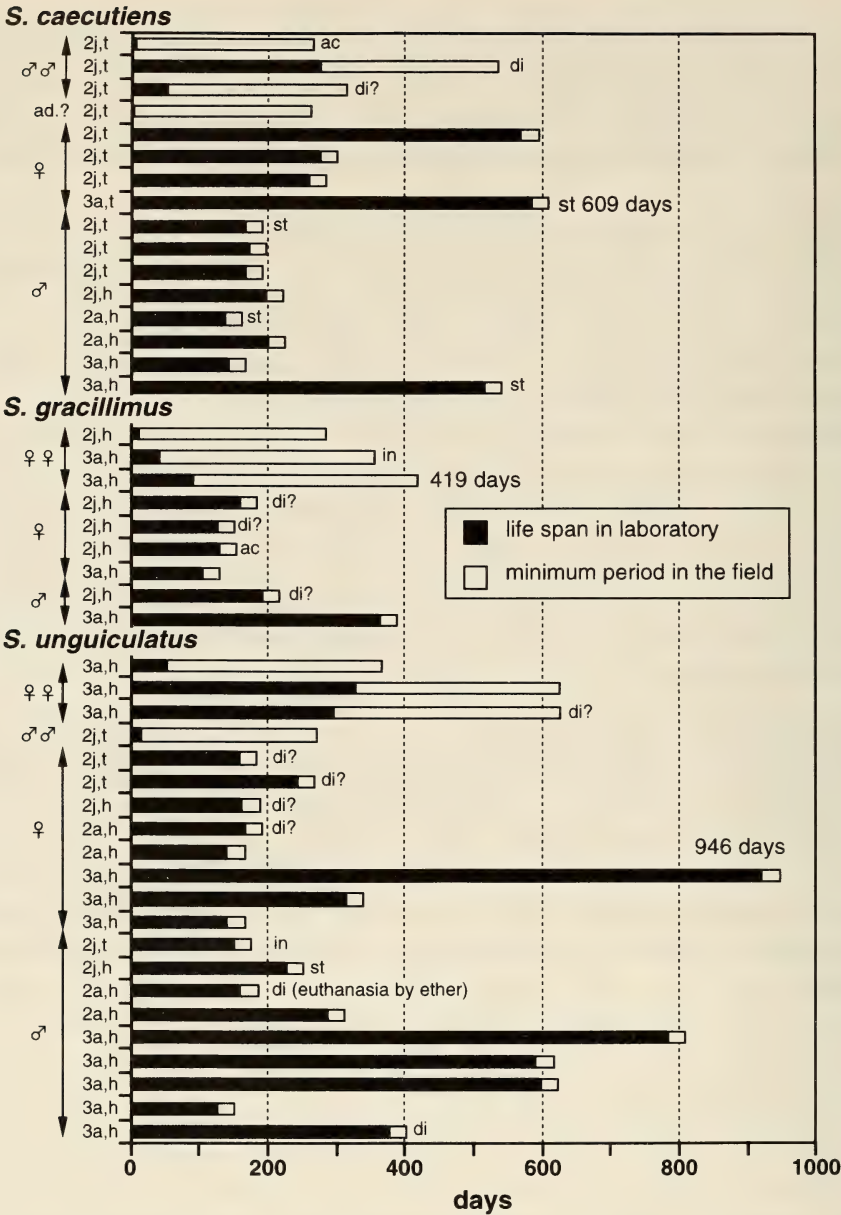
Fig. 1. The estimated longevity of three species of *Sorex* in captivity in Hokkaido. Black bars indicate the actual period shrews survived in the laboratory, and white bars indicate the estimated minimum duration in the field (see text for calculation). Young animals surviving fewer than 100 days in the laboratory were omitted from the figure. Letters to the left of the bars indicate dates of capture and localities ("2j, t" = June 1992 in Tomakomai, "2j, h" = June 1992 in Horonobe, "2a, h" = August 1992 in Horonobe, and "3a, h" = August 1993 in Horonobe). Letters to the right of the bars record the causes of death (ac = accidental kill, di = disease, st = starvation or a lack of water, in = injured, and no letter = unknown). Double sex symbols denote sexually mature animals when captured, and single ones immature animals (ad.? = sex-unknown but mature individual).

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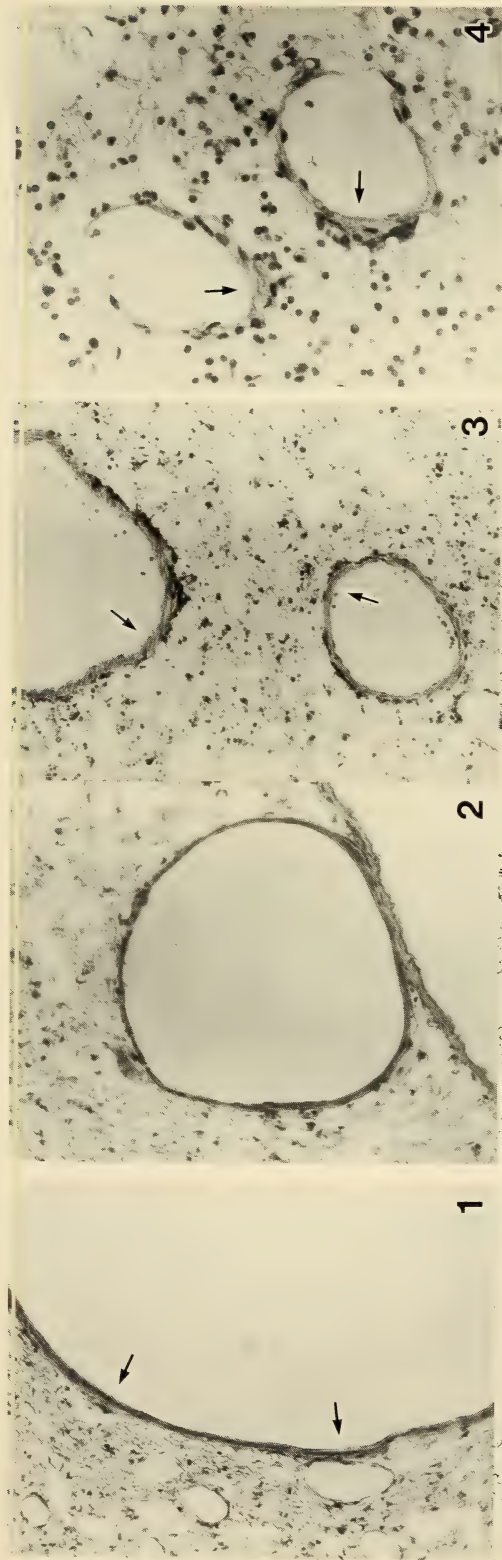# INSTRUCTIONS TO CONTRIBUTORS

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Eisenberg, J. F. 1981. The Mammalian Radiations. Univ. of Chicago Press, Chicago, 610 pp.

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Obara, Y. 1991. Karyosystematics of the mustelid carnivores of Japan. Honyurui Kagaku [Mammalian Science] 30 : 197−220 (in Japanese with English abstract).

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Mammal Study

CONTENTS

Mammal Study

The Continuation of the Journal of
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Habitat factors affecting the geographic size variation of Japanese moles

Hisashi ABE

Laboratory of Applied Zoology, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

Abstract.   Japanese moles of the genus *Mogera* show remarkable geographic variation in body size.   In order to determine which habitat factors affect them, 260 specimens of *Mogera imaizumii* were collected from 27 localities, 280 specimens of *M. wogura* were collected from 23 localities, and 41 specimens of *M. tokudae* were obtained from two localities.   The relationships between size, geographic location and seven habitat factors consisting of habitat (rice field) area, soil hardness, and five meteorological components, were analyzed.   All three species showed a positive correlation between greatest skull length and habitat area.   Populations of *M. imaizumii* from areas with heavy snow were significantly smaller than those from areas with little or no snow and this variation was also explained by the negative correlation with total annual precipitation.   In addition, the size of *M. imaizumii* varied positively with the variation in annual mean temperature.   In the correlation between skull size of *M. wogura* and habitat area, there was a significant difference in the Y-intersept between the populations from central Honshu and those from southern Honshu, Shikoku and Kyushu.   This variation was well explained by the negative correlation between skull size and mean minimum temperature.   This variation, however, was not constant across all populations examined, because *M. wogura* were smaller in narrow valleys, even where mean minimum temperatures were low.

Key words.   geographic size variation, habitat factors, *Mogera*.

Three species of *Mogera, M. wogura, M. imaizumii and M. tokudae* occur in Japan (Abe 1995, Motokawa and Abe 1996).   *M. wogura* occurs in the southern half of Honshu, in Shikoku, Kyushu and smaller islands such as Oki, Tsushima, Goto, Tanegashima and Yakushima.   *M. imaizumii* occurs mainly in the northern half of Honshu and on the small island of Awashima, but also has some scattered relic populations in certain mountainous regions in southern Honshu and Shikoku and on the small island of Shodoshima in the Inland Sea of Japan (Fig. 1).   The relic populations are surrounded by populations of *M. wogura*. *M. tokudae* is restricted to the central part of the Echigo Plain, Honshu and to Sado Island, located off the west coast of the plain.   The ranges of these three species are usually sharply segregated from each other except in some mountainous regions with very complicated topographies, as for example in Ashiu, Kyoto Prefecture, and Hiwa, Hiroshima Prefecture (Sagara *et al*. 1989, Yuka-
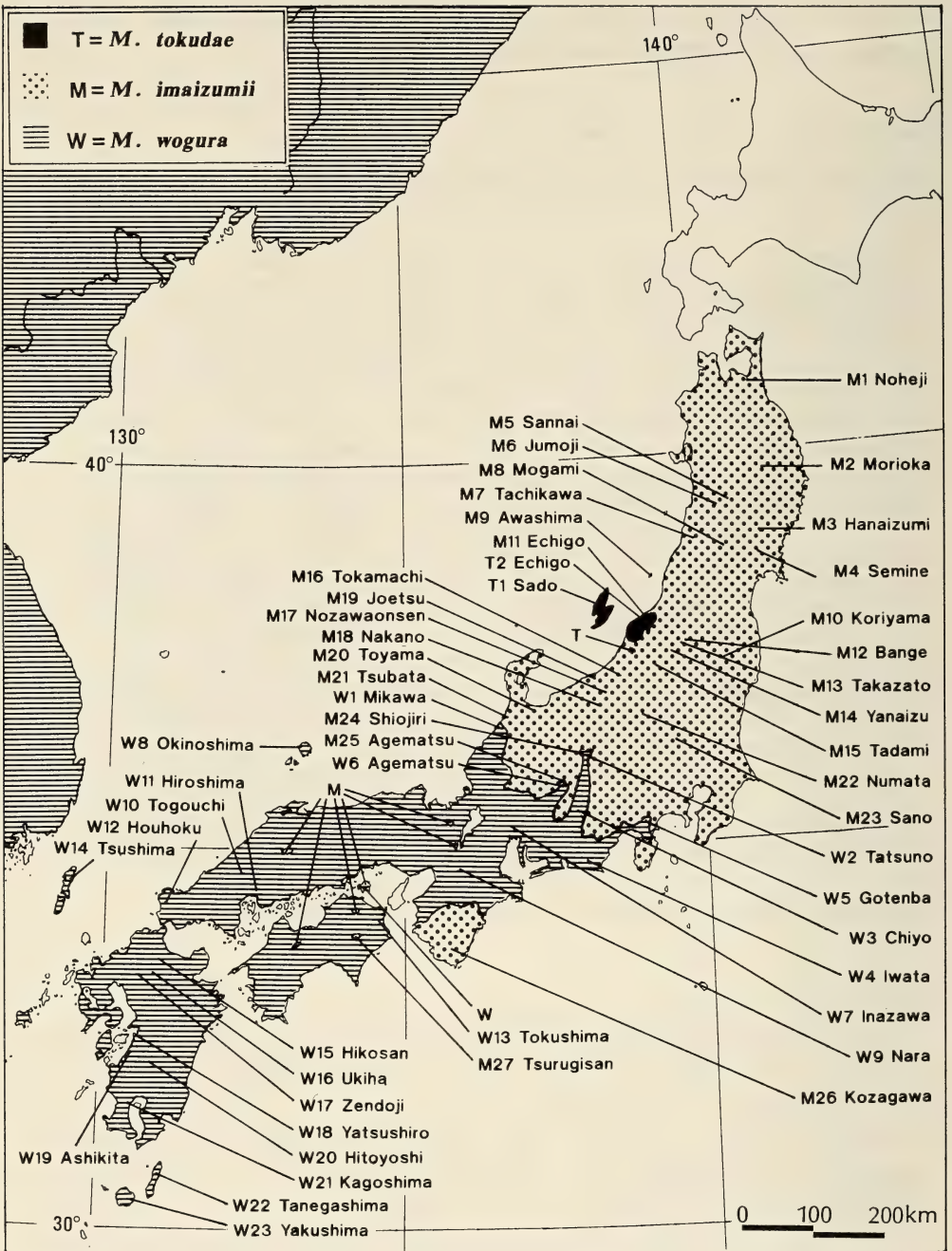
Fig. 1. The distribution and collecting sites of the three species of Japanese moles. For the locality numbers, refer to Tables 1 and 2.

wa 1977).

The Japanese moles are highly variable from range to range and in the past this has caused confusion in taxonomy (Abe 1967). Geographical variation in these moles has generally been described in relation to Bergmann's rule (Ima-izumi 1966, 1970). The variations to be observed among Japanese moles, however, are not so simple as to be totally explained by this rule. Factor analysis, based on many probable habitat factors is required for a further understanding (Abe 1967). In this study, therefore, correlation analyses between the sizes of the moles and certain habitat factors believed to influence variation, have been carried out.

MATERIALS AND METHODS

A total of 260 *M. imaizumii* were collected from 27 localities, 280 *M. wogura* were collected from 23 localities and 41 *M. tokudae* were collected from two localities (Appendix 1).

The body sizes of the specimens were expressed by the greatest skull length (GSL) measured to the nearest 0.01 mm with dial calipers. An average GSL for each local population was adopted as the representative size of moles in that population.

It has been shown empirically that the size of moles varies with habitat area, especially when the habitat consists of more or less flat fields with deep damp soft soil (Abe 1967). In Japan, this kind of habitat usually consists of rice fields, which are distributed from low alluvial plains to montane valleys. Rice fields are usually among the best habitats for moles. As an indicator of habitat size, therefore, the area (km²) of rice field in each locality was measured on a topographical map of 1/50,000 or 1/200,000. Mountains, narrow gorges and rocky slopes all represent potential barriers to mole distribution (Abe 1974, 1985); therefore the area of contiguous rice fields more or less isolated from others by such the barriers was measured, representing the size of the habitat surrounding the collecting site (Tables 1 and 2). When the collecting site was located in a montane area without any rice fields, the size of the habitat was recorded as 0.01 km².

Soil hardness (kg/cm²), an important habitat factor, was measured with an intrusive proctor needle (Daiki-rika-kogyo, DIK-5520) to a depth of 60 cm. The hardness values used in the analyses were taken every 5 cm from 12 layers (5~60 cm depth). Such measurements were taken at about 15 points for each habitat. From field surveys in Nagano Prefecture where the distributions of *M. wogura* and *M. imaizumii* are parapatric, it has been noticed that soft soils being less than 10 kg/cm² in hardness and also deeper than about 30 cm are necessary for the larger *M. wogura* to survive (Abe unpubl.). In terms of soil hardness, therefore, the deepest level at which soft soils (<10 kg/cm²) can be found at more than 50 percent of the survey points, is an important indicator of the depth of habitat appropriate for moles.

Meteorological data for the collecting localities were obtained from nearby

Table 1.   Location, sample size (N), greatest skull length (GSL), and seven habitat factors of *M. imaizumii*.

| Locality | N | GSL (mm) | Habitat area (km²) | Soft soil depth (cm) | Monthly mean temperature(°C) | | | | Annual precip. (mm) |
					Annual mean	Max. range*	Min. mean*	Max. mean	
1 Noheji	6	33.60	8.10	60	9.5	24.9	0	21.9	1420
2 Morioka	20	33.25	4.00	60	9.7	27.8	−0.66	22.9	1258
3 Hanaizumi	14	35.03	21.20	60	10.9	28.9	−0.83	23.6	1191
4 Semine	20	36.81	1150.00	60	11.0	32.5	−1.03	24.0	995
5 Sannai	3	32.73	1.80	60	8.9	24.8	0	22.6	2253
6 Jumoji	7	33.78	681.00	50	10.3	25.8	0	23.6	1612
7 Tachikawa	4	33.61	584.00	50	11.5	26.5	0	24.2	2232
8 Mogami	5	33.91	35.90	60	9.9	25.6	0	23.2	1721
9 Awashima	4	35.39	0.01		13.2	26.7	0.59	25.1	1751
10 Koriyama	8	36.11	734.00	45	11.9	28.5	0.24	25.1	1112
11 Echigo	16	33.25	1671.00	60	12.9	27.5	0.48	25.5	1958
12 Bange	14	34.70	330.00	60	11.1	26.7	−0.10	24.3	1129
13 Takazato	15	34.04	8.40	45	11.1	26.8	0	24.4	1789
14 Yanaizu	14	33.19	2.50	30	10.5	26.5	0	23.8	2021
15 Tadami	14	32.71	1.20	50	10.1	25.8	0	23.4	2268
16 Tokamachi	9	34.01	149.10	60	11.6	27.0	0	25.0	2655
17 Nozawaonsen	2	33.26	5.10	60	10.5	25.8	0	23.8	1955
18 Nakano	16	35.76	298.00	60	10.8	26.6	0	24.6	1538
19 Joetsu	6	33.81	320.00	60	13.1	24.1	0	25.6	2857
20 Toyama	4	35.64	1061.00	60	13.4	27.3	0	25.7	2182
21 Tsubata	4	33.89	457.00	60	14.0	28.0	0	26.2	2498
22 Numata	7	35.94	33.80	55	11.3	26.5	0.36	23.8	1041
23 Sano	4	36.58	1000.00	60	13.7	28.0	2.14	25.6	1186
24 Shiojiri	21	34.88	3.70	60	11.1	28.7	−0.89	23.8	1018
25 Agematsu	16	33.79	1.90	30	10.4	27.3	−0.78	22.8	1989
26 Kozagawa	3	33.31	0.06		14.7	24.3	3.74	25.5	3616
27 Tsurugisan	4	31.69	0.01		4.2	16.8	0	15.3	2814

*In localities with continuous heavy snow cover (>25 cm in depth) during winter, the average temperature at ground level was adjusted to a constant 0°C in those months.

Table 2. Location, sample size (N), greatest skull length (GSL), and seven habitat factors of *M. wogura* and *M. tokudae*.

Locality	N	GSL (mm)	Habitat area (km²)	Soft soil depth (cm)	Monthly mean temperature(°C) Annual mean	Max. range*	Min. mean*	Max. mean	Annual precip. (mm)
M. wogura									
1 Mikawa	4	39.95	456.80	60	14.0	27.9	0.00	26.2	2262
2 Tatsuno	13	41.49	252.80	55	10.4	26.5	−1.01	22.5	1535
3 Chiyo	15	39.26	0.43		8.8	23.6	−1.63	20.1	2730
4 Iwata	9	41.17	410.40		15.6	24.3	5.01	26.3	1950
5 Gotenba	7	40.68	38.40		12.5	24.7	2.09	23.4	2908
6 Agematsu	26	38.90	1.30	60	11.5	26.9	0.18	23.5	2689
7 Inazawa	15	39.83	1000.00	30	14.2	25.5	2.57	26.1	1842
8 Oki	30	39.77	2.80		13.7	27.1	2.19	25.2	1640
9 Nara	5	40.19	401.00	45	14.3	26.1	3.07	26.2	1354
10 Togouchi	15	37.04	6.90	25	13.1	27.1	0.68	25.4	1865
11 Hiroshima	16	38.37	86.50		14.9	25.9	3.85	26.6	1476
12 Houhoku	1	34.89	3.70		15.0	25.0	4.66	26.1	1794
13 Tokushima	14	38.74	344.80		16.6	24.2	6.52	26.9	3257
14 Tsushima	8	35.34	2.00		14.9	25.9	4.28	26.1	2045
15 Hikosan	5	36.01	0.38	25	10.6	26.8	0.08	22.6	2469
16 Ukiha	12	37.06	44.60	20	15.4	26.7	4.03	25.5	1868
17 Zendoji	13	38.08	1200.00	20	15.8	26.4	4.44	27.6	1791
18 Yatsushiro	9	37.39	453.70	60	16.4	25.5	5.68	27.6	2002
19 Ashikita	9	36.15	6.80	30	16.3	22.9	6.06	26.6	1968
20 Hitoyoshi	8	36.58	142.70	45	14.9	25.2	3.27	25.9	2309
21 Kagoshima	11	35.06	0.20	60	17.5	23.8	6.61	27.9	2217
22 Tane	18	34.41	1.20		19.2	19.8	10.87	27.5	2254
23 Yaku	17	35.21	0.24		19.1	20.4	10.68	27.2	3959
M. tokudae									
1 Sado	17	39.18	104.40		13.0	26.5	0.44	25.1	1867
2 Echigo	24	41.13	1671.00	60	12.9	27.5	0.48	25.5	1958

*Refer to Table 1.

meteorological observatories or stations. Mean values for the 16 years from 1967 to 1982 were used (Takahashi 1983) except for those from Hikosan where data from 1980 to 1994 were used. Data collected included: the annual mean temperature; the maximum annual range of monthly mean temperatures; the mean of monthly minimum temperatures; the mean of monthly maximum temperatures, and the mean of total annual precipitations. In localities with continuous heavy snow cover (>25 cm in depth) during the winter months, the average temperature at ground level was adjusted to a constant 0°C, irrespective of ambient temperatures recorded during those months. Fifteen localities in northwest Honshu and on Mt. Tsurugisan (alt. 1995 m), Shikoku, are located in area with heavy snow falls.

A multiple or simple regression analysis was performed to detect the relationships between size variation of moles and habitat factors. ANCOVA test was used for the intraspecific comparison of geographic variations between two groups of localities, and Mann-Whitney's U-test was used for the comparison of two samples with different variances.

RESULTS

Japanese moles are highly variable geographically (Fig. 2). The two main species, *M. imaizumii* and *M. wogura*, however, differ in their trends of latitudinal variation. A multiple regression analysis between GSL, a dependent factor, and seven independent habitat factors: the log-transformed area of habitat; the log-transformed depth of soft soil (except for *M. wogura*, where many localities lacked data); the annual mean temperature; the maximum annual range of monthly mean temperatures; the mean of monthly minimum temperatures; the mean of monthly maximum temperatures, and the mean of annual precipitation. The regression was significant for both *M. imaizumii* ($R^2 = 0.734$, $F = 6.317$, $p = 0.0011$) and *M. wogura* ($R^2 = 0.692$, $F = 5.991$, $p = 0.0019$). The mean of annual precipitation ($p = 0.0106$) for *M. imaizumii* and habitat area ($p = 0.0007$) for *M. wogura* were significantly correlated with size (Table 3). In *M. imaizumii*, the size of Echigo specimens was considerably smaller than all others, probably, as discussed later, due to factors other than habitat and as a consequence the regression model was not significant ($p = 0.1804$). When recalculated ignoring the data from Echigo, the regression was refined ($p < 0.0001$), and the correlation between mole size and habitat area became significant ($p = 0.0496$). Thus the sizes of both species varied positively with habitat area.

Based on a stepwise regression analysis, the crucial habitat factors were reduced to: habitat area, annual mean temperature, and annual precipitation in *M. imaizumii* ($R^2 = 0.797$, $F = 28.839$, $p < 0.0001$), and habitat area and mean maximum temperature in *M. wogura* ($R^2 = 0.682$, $F = 21.448$, $p = 0.0001$).

For a detailed examination of the variation in some local mole populations, further analyses were carried out for habitat factors selected above.

Populations of *M. imaizumii* from areas with heavy snow appeared to

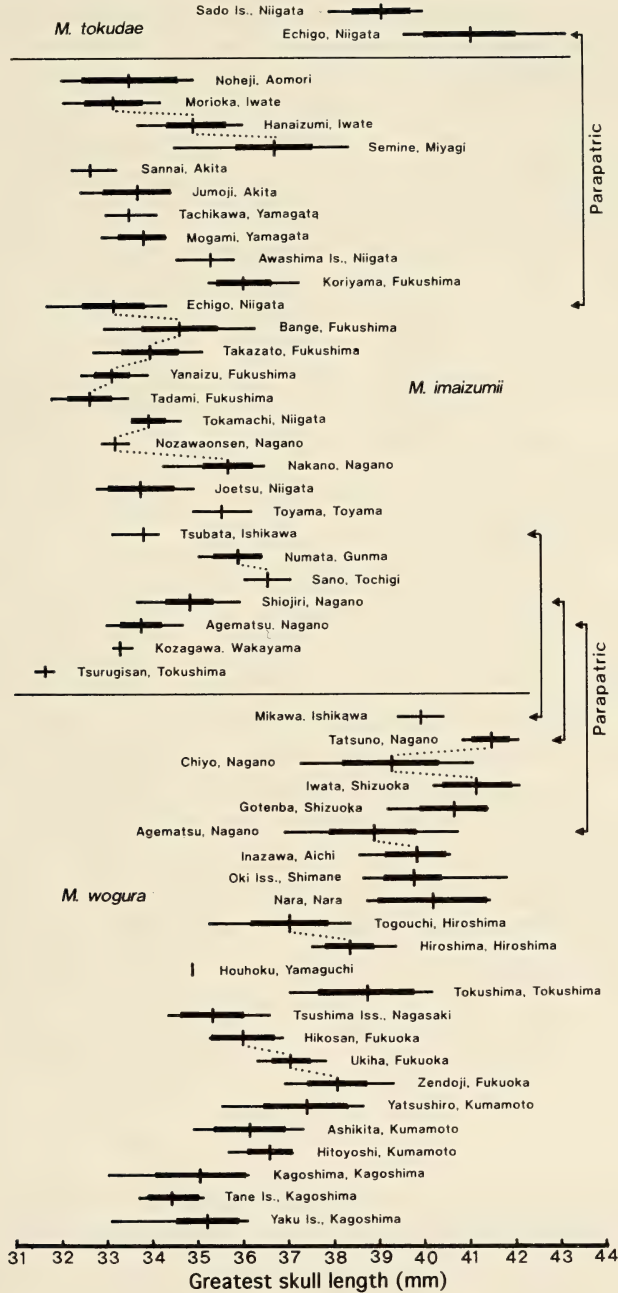Fig. 2. Geographic variation in greatest skull length of three species of Japanese moles. Localities are arranged from south (lower) toward north (upper) for each species and those connected with dotted lines are localities situated along a river basin. The horizontal line indicates the total variation of the sample; the broad portion of the line, one standard deviation on each side of the mean; the vertical line, the mean.

Table 3. Results of the regression analyses between greatest skull length and habitat factors. C. coefficient, SC. standard coefficient.

(1) Multiple regression analysis

	C.	SC.	p-value	C.	SC.	p-value
M. imaizumii	All populattions			Sample excluding Echigo pop.		
Ann. mean temp.	0.127	0.141	0.7164	0.154	0.169	0.5782
Max. range temp.	0.133	0.187	0.4000	0.165	0.235	0.1988
Mean min. temp.	0.057	0.028	0.8793	0.151	0.076	0.6143
Mean max. temp.	0.164	0.148	0.7238	0.131	0.116	0.7218
Ann. precip.	−0.001	−0.581	0.0106	−0.001	−0.538	0.0047
Log area	0.300	0.286	0.1804	0.364	0.340	0.0496
Log soil depth	−0.278	−0.020	0.8865	−0.019	−0.001	0.9904
Intercept	27.545		0.0050	26.529		0.0013
M. wogura	All populations					
Ann. mean temp.	0.189	0.224	0.8512			
Max. range temp.	−0.168	−0.156	0.6647			
Mean min. temp.	−0.317	−0.474	0.5701			
Mean max. temp.	−0.512	−0.457	0.5467			
Ann. precip.	0.0001	0.055	0.7645			
Log area	1.234	0.725	0.0007			
Intercept	51.609		<0.0001			

(2) Simple regression analysis

	C.	SC.	p-value	C.	SC.	p-value
M. imaizumii	Heavy snow area's populations			Little or no snow area's populations.		
Log area	0.509	0.717	0.0012	0.543	0.742	0.0140
Intercept	32.932		<0.0001	34.620		<0.0001
M. imaizumii	All populations			All populations		
Ann. precip.	−0.001	−0.623	0.0005			
Ann. mean temp.				0.328	0.517	0.0068
Intercept	36.478		<0.0001	30.643		<0.0001
M. wogura	Northern populations			Southern populations		
Log area	0.422	0.637	0.0651	0.904	0.834	0.0002
Intercept	39.432		<0.0001	35.490		<0.0001
M. wogura	All populations			All populations		
Mean min. temp.	−0.388	−0.580	0.0037			
Mean max. temp.				−0.446	−0.398	0.0598
Intercept	39.313		<0.0001	49.326		<0.0001

differ in the relationship between GSL and log-transformed habitat area from those from little or no snow areas (Figs. 3 and 4). A simple regression analysis revealed significant regressions for the two groups (heavy snow: $R^2 = 0.515$, $F = 15.913$, $p = 0.0012$; little or no snow: $R^2 = 0.550$, $F = 9.791$ $p = 0.0140$). Furthermore, an ANCOVA test revealed a highly significant difference in Y-intercept between the two groups ($p < 0.001$; regression coefficient: $p = 0.485$). In *M. wogura*, the same analysis was made comparing northern (Nara-Oki Island and northern ones) and southern populations (southern Honshu, Shikoku and Kyushu). In the northern populations, no significant regression was observed ($R^2 = 0.406$, $F = 4.776$, $p = 0.0651$), while in the southern population it was significant ($R^2 = 0.696$, $F = 27.512$, $p = 0.0002$). An ANCOVA test showed a significant difference in the Y-intercept between the two groups ($p < 0.001$; regression coefficient: $p = 0.156$).

Simple regression analyses suggest that the size of *M. imaizumii* decreased as annual precipitation increased (regression coefficient = -0.001; $R^2 = 0.388$, $F = 15.844$, $p = 0.0005$) and varied positively as annual mean temperature increased (regression coefficient = 0.328; $R^2 = 0.268$, $F = 8.776$, $p = 0.0068$) (Figs. 5 and 6). In *M. wogura*, the simple regression analysis between GSL and mean monthly maximum temperature showed an insignificant relationship ($R^2 = 0.159$, $F = 3.958$, $p = 0.0598$), whereas a significant relationship between GSL and mean monthly minimum temperature was indicated ($R^2 = 0.337$ $F = 10.658$, $p = 0.0037$; Fig. 7). Thus, the size of *M. wogura* increased as mean monthly minimum temperature decreased, with a regression coefficient of -0.388 (Table 3). Other factors were not significant for this species.

There are only two major populations of *M. tokudae* and these are isolated on Sado Island, and on the Echigo Plain, Honshu, both of which experience very similar climatic conditions (Table 2). Consequently, geographic variation in relation to meteorological factors could not be analyzed in detail. The relationship between GSL and habitat area for the two populations of *M. tokudae*, however, resembled those in the former two species; *M. tokudae* from the larger Echigo Plain were significantly larger than those from Sado Island (Mann-Whitney U-test, $p < 0.0001$).

DISCUSSION

In all three species of Japanese moles, geographic variation in size as indicated by GSL was significantly correlated with habitat area, such that size increased as habitat area increased. *M. imaizumii* and *M. wogura* differed somewhat, however, in their reaction to habitat factors with *M. imaizumii* responding differently to habitat area in regions of heavy snow, and in regions with little or no snow, a difference which could be attributed to a correlation with annual precipitation. Toyama, Tsubata, Tokamachi and Joetsu populations, all in areas experiencing heavy snow falls close to the Japan Sea, were all relatively large in comparison with from inland localities with heavy snow but narrow areas of habitat, *e. g.* Takazato, Sannai, and Tadami (Fig. 5). The
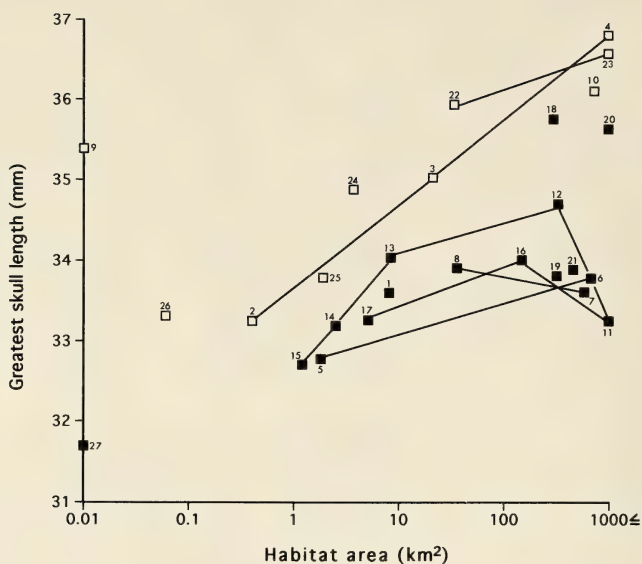
Fig. 3.   The relationship between greatest skull length and habitat area (log scale) in *M. imaizumii*.   Solid marks indicate samples from heavy snow areas ; open ones, those from little or no snow areas.   Localities connected with lines are those situated along a river basin. Numbers at each mark are those of localities in Table 1.
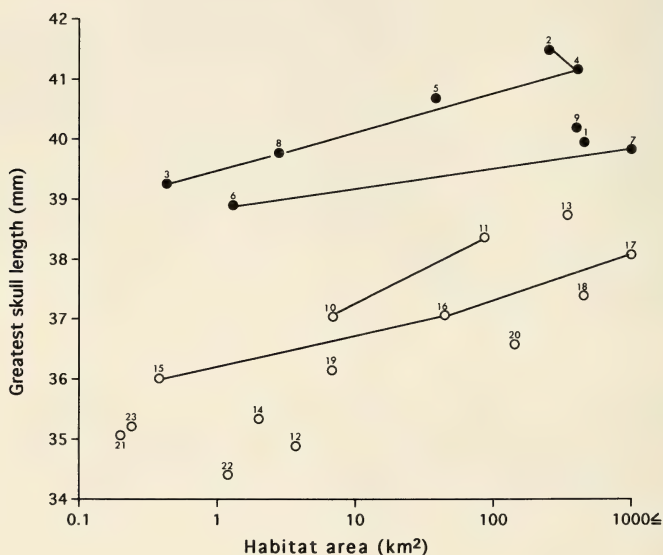
Fig. 4.   The relationship between greatest skull length and habitat area (log scale) in *M. wogura*.   Solid marks indicate samples from Nara-Oki and northern populations ; open ones, those from southern Honshu, Shikoku and Kyushu.   Refer to Fig. 3 and Table 2 for other legends.
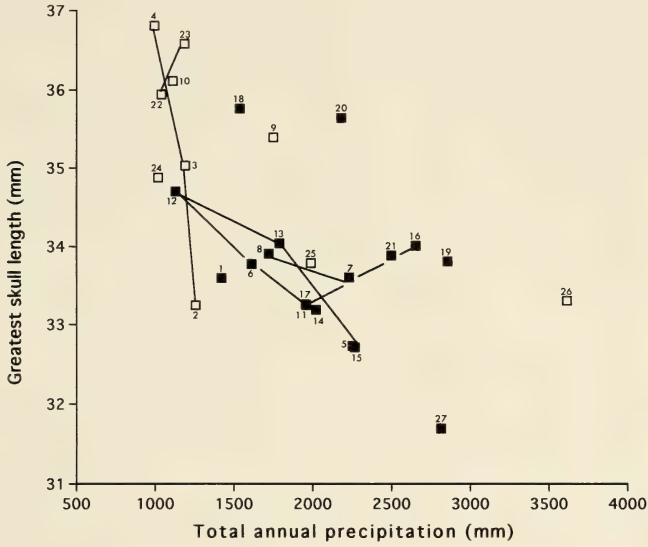
Fig. 5.   The relationship between greatest skull length and total annual precipitation in *M. imaizumii*.   Refer to Fig. 3 for legends.
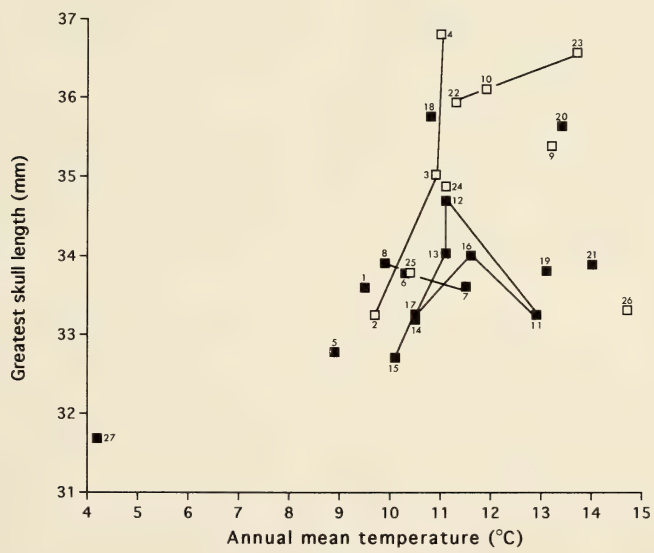
Fig. 6.   The relationship between greatest skull length and annual mean temperatures in *M. imaizumii*.   Refer to Fig. 3 for legends.
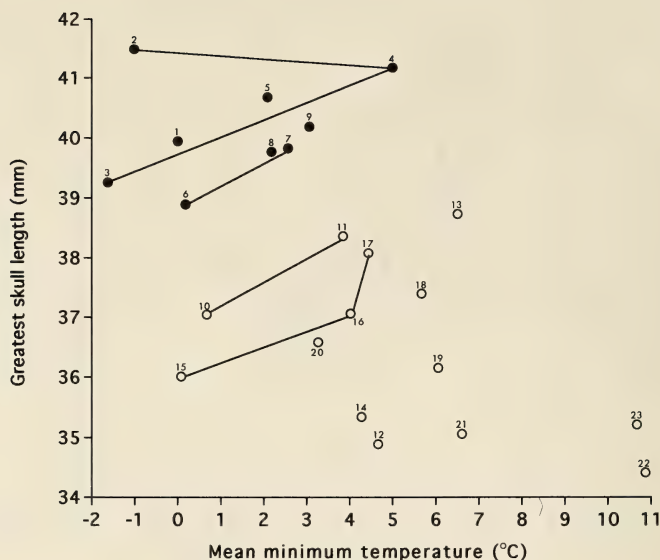
Fig. 7.   The relationship between greatest skull length and mean monthly minimum temperatures in *M. wogura*.   Refer to Fig. 4 for legends.

populations from Awashima and Kozagawa where continuous snow cover does not occur in winter were also relatively large (Fig. 5).   Of these, the former may be explained by the high annual mean temperature affected by the Tsushima Warm Current, while the latter could not be well accounted for by this factor.   The exceptional size of the Awashima population in Fig. 3 may also be attributable to the same factor.

The size of *M. imaizumii* varies positively with annual mean temperatures (Fig. 6), and the size variation indicates a reverse of Bergmann's rule.   In this case, the populations of Echigo, Joetsu, Tsubata, and Kozagawa are relatively small.   The reasons for this are not known, but they may differ between the former three and the last, because of the great difference in habitat areas between them.

Thus, some local *M. imaizumii* populations differed in body size from the general trend.   One of the most remarkable variations from the general trend was found in the population of Echigo, followed by those of Tsubata and Joetsu (Figs. 3 and 6).   The most remarkable aspect of the habitat in Echigo is that two species, *M. imaizumii* and *M. tokudae* occur there, and the former are very small while the latter are very large (Fig. 2); thus the biotic situation in this habitat is different from most of the others.   Interspecific competition in moles appears to be so severe that in plains with simple topographies such as at Echigo, two species of moles never have overlapping ranges and are strictly parapatric (Abe 1974, 1985).   In the Echigo Plain, *M. imaizumii* and *M. tokudae* are clearly parapatric, consequently, the extremely small size of *M. imaizumii* there cannot be attributed to a change in size due to character displacement

(Brown and Wilson 1956) which is a common biological mechanism serving to reduce competition between ecological equivalents. One further interesting aspect of this case, is that the larger species, *M. tokudae*, is actually retreating, and reducing its original distribution, while *M. imaizumii*, despite its smaller body size, is invading the habitat of *M. tokudae* and expanding its range on the plain (Imaizumi and Imaizumi 1970, Abe unpubl.). From these facts, it is plausible to hypothesize that the extremely small *M. imaizumii* of the Echigo are recent newcomers, in the geological or evolutionary sense, having immigrated from the surrounding, small-bodied mountain populations. Probably they are moles that have not yet fully adapted to the high quality habitat, which typically results in larger-bodied moles.

In the south of its main range of *M. imaizumii* in Honshu, there are three known populations which abut those of *M. wogura*, another large species, at Tsubata, Agematsu and Shiojiri (Kita-ono) (Fig. 1). In these areas, however, *M. imaizumii* is retreating as *M. wogura* is expanding its range (Abe 1974, 1985). Agematsu and Shiojiri (Kita-ono) are located along the uppermost reaches of the Kiso and Tenryu rivers, respectively. At both these sites *M. imaizumii* remain reasonable sizes with respect to the size of the respective habitats (Figs. 3, 5, 6). *M. imaizumii* at Tsubata, another population confronting *M. wogura*, are somewhat smaller than might be expected in proportion to habitat area and annual mean temperature. The reason for this, however, is not known.

In *M. wogura* the relationship between body size and habitat area differs between the northern and southern populations (Fig. 4), but as a whole body size increases as mean monthly minimum temperatures decline, a variation which coincides with Bergmann's rule (Fig. 7). When examined on a smaller scale, however, size variation in each group of sites along a river basin showed the reverse tendency, with body size decreasing as temperatures decreased along the upper reaches of rivers (Fig. 7). This aspect of size decrease in *M. wogura*, consequently, may be accounted for by the effect of reduced habitat area at such locations. When studying size variation in this species, therefore, samples should only be compared with those from habitats of a similar size.

The *M. wogura* populations of Tatsuno, Agematsu and Mikawa are parapatric with those of *M. imaizumii* and are expanding northwards, replacing those of the latter (Abe 1974, 1985). In these three areas, only the moles of Tatsuno are relatively larger than the others, probably as a result of the compounded effect of the relatively wide habitat area in the Ina Valley, where Tatsuno is located, and the lower monthly minimum temperature (Figs. 4 and 7). Although the moles of Mikawa and Agematsu experience similar monthly minimum temperatures, the former are slightly larger than the latter, perhaps accounted for by the wider habitat at Mikawa.

The moles of Chiyo and Agematsu are large relative to the restricted areas of habitats available. This may be explained taking the same perspective as that of *M. imaizumii* in the Echigo Plain, that is they are recent immigrants from populations of very large moles such as from the Iwata-Tatsuno populations for the Chiyo moles, and from the Inazawa population for the Agematsu

moles, both of which represent the expanding nothernmost frontier populations of *M. wogura*. The extraordinarily large size of the moles in these two populations may be the main reason for the insignificant correlation between size and habitat area only in the northern populations mentioned above (Fig. 4). Thus, it is expected that the moles of these two populations will decrease in body size in the future to a level reasonable for the habitat area.

It is interesting that the populations of the species showing extraordinary variation, irrespective of whether they are larger or smaller, at the contact point between areas occupied by two species are not original residents of the area but immigrants. Thus, whereas the original residents are reasonably proportioned in relation to their habitat as a result of evolutionary or historical adaptation, while the immigrant population has not yet attained the optional size for the habitat, and still retain, in their new habitat, their original size related to their original native habitats. This is the most plausible explanation for the extraordinary sizes of moles observed at the expanding edge of their ranges.

Soil hardness has been considered to be an important limiting factor for the fossorial life of moles (Abe 1974); however, in this study it was not found to be significantly correlated with variation in body size. This might be a natural consequence of moles usually preferring habitats with deep soft soils within their range and because hardness was measured precisely in habitats preferred by the moles. At Kita-ono, Shiojiri City, Nagano Prefecture, for example, the range of *M. wogura* reaches its northernmost frontier along the uppermost tributary of the Tenryu River. The range expansion of this species has been blocked since at least 1959 when I first surveyed the area, by the shallow hard soil surrounding the present habitat, which is confined here only to narrow zones of soft soil along the banks of small streams (Abe 1985 and unpubl. data). This type of localized habitat preference may result in an apparent non-relationship between soil hardness and mole body size as in the present analysis.

Boyce (1979) presented a hypothesis in which the seasonality of habitat aspects was a very important factor in the evolution of large body size in homeothermic vertebrates. In the present study, the maximum annual range of monthly mean temperatures was one of the factors, but it was not significant in the variation of *M. wogura* and *M. imaizumii*.

Much work has been devoted to body size variation of mammals on islands, and several hypotheses have been presented (Foster 1964, Heaney 1978, Lawlor 1982, Angerbjorn 1985, Lomolino 1985, Abe and Ishii 1987). There are, however, still no concrete hypotheses to explain all the size variations on islands. In the present study of moles from the Japanese islands, no definite tendency in size variation was observed. In *M. wogura*, for example, variation between islands was basically explained by habitat area or by mean minimum temperatures (Figs. 4 and 7); however, the Awashima population of *M. imaizumii*, was considerably larger than all others, in relation to habitat area. This is considered to be the effect of the warm climatic conditions on Awashima, on the general tendency of size variation in this species.

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APPENDIX 1

Specimens examined
All the speimens used in this work were collected by the author.   Locality, with
the third mesh (*ca.* 1×1 km²) code number (LC no.) of the Environment Agency,
Japan, the month and year of collection, and the registration number (Hok-
kaido University Abe's collection number : A no.) of all specimens examined
are listed below.

M. imaizumii
1) Noheji T., Aomori Prf. LC6141-21-10~20, October 1959, A3028~30, August
1993, A5811~12, Tenmarin V., LC6141-01-74, August 1993, A5813.   2) Morioka
C., Iwate Pref.: Kuroishino LC5941-41-81, October 1959, A3031~35;
Kamiyonai LC5941-41-96, October 1959, A3036~45; Asagishi LC5941-41-58,
October 1959 A3046~50.   3) Hanaizumi T., Iwate Pref. LC5841-11-95, July
1960, A3394~3407.   4) Semine T., Miyagi Pref. LC5741-70-76, July 1960, A3374
~93.   5) Sannai V., Akita Pref. LC5840-75-26, August 1993, A5883~85.   Jumo-
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18) Nakano C., Nagano Pref. LC5538-02-66 and 76, August 1991, A5758~73.
19) Joetsu C., Niigata Pref. LC5538-32-52 and 83, August 1991, A5750~55.
20) Toyama   C.,   Toyama   Pref.   LC5537-01-24,   August   1993,   A5850~53.
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Morphological variation, and latitudinal and altitudinal distribution of *Eothenomys chinensis, E. wardi, E. custos, E. proditor,* and *E. olitor* (Rodentia, Arvicolidae) in China

Yukibumi KANEKO

Biological Laboratory, Faculty of Education, Kagawa University, Takamatsu 760, Japan
Fax. 0878-36-1652

Abstract. A total of 308 museum specimens of the genus *Eothenomys* from five separate areas in Sichuan (Szechwan) and Yunnan Provinces, China, were categorized by the relationship between condylobasal length (CBL) and tail length (TL). These specimens were allocated to three larger species, *E. chinensis, E. wardi* and *E. proditor,* and two smaller ones, *E. custos* and *E. olitor.*
    E. chinensis and *E. wardi* are allopatric, and their distributions separated by about 240 km in northern high mountain areas (28–30° N). *E. chinensis* lives at altitudes above 1500 m, whereas *E. wardi* was found above 2300 m. Lengths of bulla (BL), tail (TL) and hind foot (HFL) were slightly larger in *E. chinensis* than in *E. wardi*
    E. custos has a large latitudinal range between 26° and 29° N in Sichuan and Yunnan Provinces, whereas *E. proditor* occurs near the borders of Sichuan and Yunnan (27–28° N). The latitudinal range of *E. custos* overlaps with that of *E. proditor* in the areas of 26–28° N and 100–102° E, but *E. custos* was found at slightly higher altitudes (2500–4800 m) than *E. proditor* (2500–4200 m).
    The distance between the anterior-most point on the upper incisor to the posterior-most edge of the third upper molar (I–M3) and BL of *E. custos* tended to increase from south to north, whereas those of *E. proditor* tended to decrease. *E. custos* had longer tails in localities around 29° N and 101.5° E than in other areas.
    E. olitor was recorded from two widely separated localities (*ca.* 23.5° N and 99.5° E, and *ca.* 27° N and 104° E).

Key words : distribution, *Eothenomys*, identification, southwest China, taxonomy.

The classification and identification of the genus *Eothenomys* (Rodentia, Arvicolidae) have remained confused, because no study on morphological variation has been carried out over the entire geographical range of the genus. Furthermore, a number of nominated species have all been identified as *Clethrionomys rufocanus* (Hinton 1926, Allen 1940, Tokuda 1941, Ellerman 1941,

Ellerman and Morrison-Scott 1951, Jones and Johnson 1965, Gromov and Polyakov 1977). Kaneko (1990, 1992) has already documented the morphological variation, identification, and geographical distribution of *E. regulus, E. shanseius, E. inez,* and *E. eva* on the Korean Peninsula and in northern and central China, all of which proved to be distinct from *C. rufocanus.*

The classification of, and keys for the identification of other species of *Eothenomys* living in central and southern China, Taiwan, Vietnam, Thailand, Burma, and India, have not been well established yet, and only crude distribution maps have been provided (Allen 1940, Corbet 1978, Corbet and Hill 1992).

In Sichuan and Yunnan Provinces, China, with the exception of the *E. melanogaster* group (which includes *fidelis, eleusis,* and *miletus*), some taxonomists recognize four species of *Eothenomys* (*chinensis, custos, proditor* and *olitor*) (Allen 1940, Ellerman and Morrison-Scott 1951, Corbet 1978, Honacki *et al.* 1982, Corbet and Hill 1991, Musser and Carleton 1993), whereas others recognize five (*chinensis, wardi, custos, proditor* and *olitor*) (Hinton 1926, Ellerman 1941, Gromov and Polyakov 1977, Corbet and Hill 1992).

The purpose of this paper is to describe identification methods and to establish the geographical distribution of *Eothenomys* spp. in Sichuan and Yunnan Provinces, China, based on the morphological variation in external and skull measurements, and in molar characteristics.

MATERIALS AND METHODS

A total of 308 specimens were examined in the following institutions : the Natural History Museum, London (BM) ; the United States National Museum of Natural History (USNM) ; the American Museum of Natural History (AMNH) ; the Museum of Comparative Zoology, Harvard University (MCZ) ; the Field Museum of Natural History (FMNH) ; the Zoological Institute, Academia Sinica (ASZI) ; and the Kunming Institute of Zoology, Academia Sinica (ASKZI).

The localities from which specimens were collected, and their reference numbers, are shown in Fig. 1, while the latitude, longitude, altitude, date collected, museum and registration number of all specimens examined can be found listed in the Appendix. The latitude and longitude of each locality were determined from gazetteers (Zhuang 1983, Su 1984) and from accounts of collecting expeditions (Kingdon Ward 1923, Roosevelt and Roosevelt 1929). Altitudes and distances were obtained from labels attached to specimens, and those recorded in feet and miles were converted to meters and kilometers. Some of these specimens had previously been described or identified by other researchers (Thomas 1891, 1911a, b, 1912a, b, 1914, 1923, Miller 1896, Allen 1912, 1924, 1940, Hinton 1923, 1926, Howell 1929, Osgood 1932, Pen *et al.* 1962, Lu *et al.* 1965).

It is difficult to appreciate the variation among these vole species at first glance, because of the great variation among the 42 localities from which they were collected. These localities were grouped into five geographical areas :
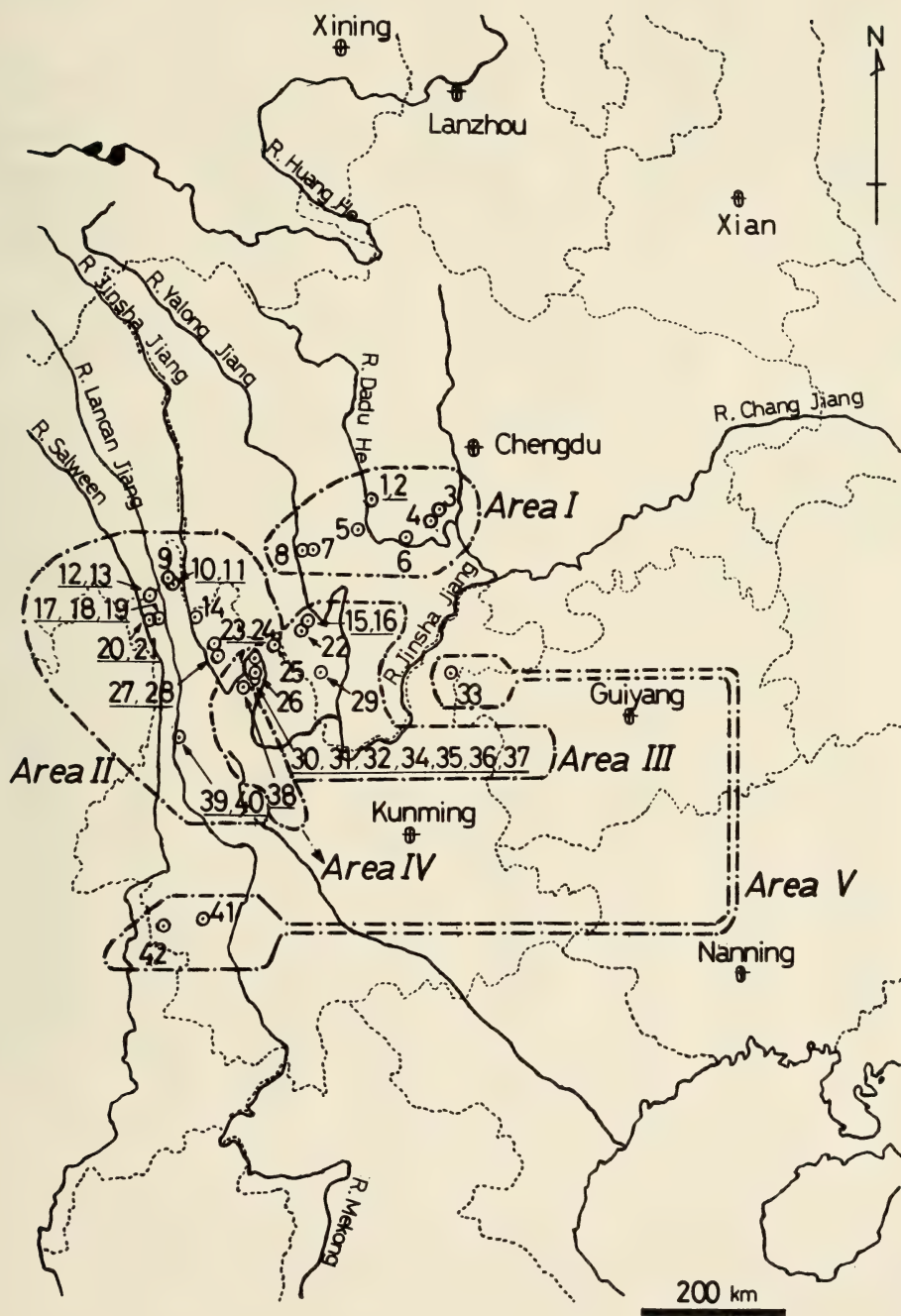
Fig. 1.   Sichuan and Yunnan Provinces, China, showing Localities 1–42 grouped into Areas I–V, as defined in this study.

Area I, Localities 1–8; Area II, Localities 9–14, 17–21, 23–24, 27–28, and 39–40; Area III, Localities 15–16, 22, 25–26, 29–32, and 34–37; Area IV, Locality 38; and Area V, Localities 33, and 41–42. Locality 38 (the Lichiang Range) was divided into ten different altitudinal zones.

Measurements of head and body length (H & BL), tail length (TL), and hind foot length (HFL), were obtained from labels attached to specimens. The presence of mammae was checked for on the skins of females. Condylobasal length (CBL), incisor-third upper molar length (I–M3), condyle-first upper molar length (C–M1), the length of bulla (BL), and the interorbital width (IOW), were measured to the nearest 0.1 mm with a dial caliper by the author (the minimum accuracy=0.05 mm).

These measurements are defined as follows: the CBL is the distance between the occipital condyle and the anterior point of the premaxillae; I–M3 is the distance from the anterior-most point on the upper incisor to the posterior-most edge of the third upper molar; C–M1 is the distance between the occipital condyle and the anterior edge of the first upper molar; BL is the longest length of the auditory bulla, and IOW is the shortest measurement of the frontal bones between the orbits.

Where specimens skulls had been damaged, CBL was estimated from regression lines between I–M3 and CBL or between C–M1 and CBL, using data from specimens with undamaged skulls. The regression lines were calculated separately for four geographical areas: Area I ($n=49$) CBL=1.492(I–M3)+2.644, CBL=1.482(C–M1)+1.171; Area II ($n=45$) CBL=1.551(I–M3)+1.514; Area III ($n=31$) CBL=1.537(I–M3)+1.543, CBL=1.693(C–M1)−1.955; and Area IV ($n=64$) CBL=1.422(I–M3)+3.193, CBL=1.671(C–M1)−1.596. Regression coefficients of these lines ranged from 0.906 to 0.982 ($p<0.05$).

Specimens were identified as adult by the presence of mammae, or as young by the presence of minute skull perforations and the absence of full ossification.

Enamel patterns on the occlusal surfaces of the upper molars, were drawn from pictures taken of the molar rows using a Nikon SMZ–10 stereo microscope at 6.6× magnification. Original close-up photographs were taken of the museum specimens using an accessory close-up lens (1.75× magnification) attached to an Olympus camera. The enamel patterns on the third upper molar were classified into five types (A–E; see Fig. 2). Type A has three salient and two re-entrant folds on the lingual side. It also has a posterior loop in which the inner enamel lamellae has either a straight or concave outline which does not protrude posteriorly beyond line "h" which crosses perpendicularly to the longitudinal axis of the tooth on the lingual side of the posterior loop (Fig. 2); Type B has four salient and three re-entrant folds on the lingual side, where the base line of the enamel lamellae of the third re-entrant fold protrudes beyond line "h"; Type C has four salient and four re-entrant folds with a posterior loop where the inner enamel lamellae has either a straight or concave outline but does not protrude line "h" (compared with Type A); Type D has five salient and four re-entrant folds on the lingual side where the outline
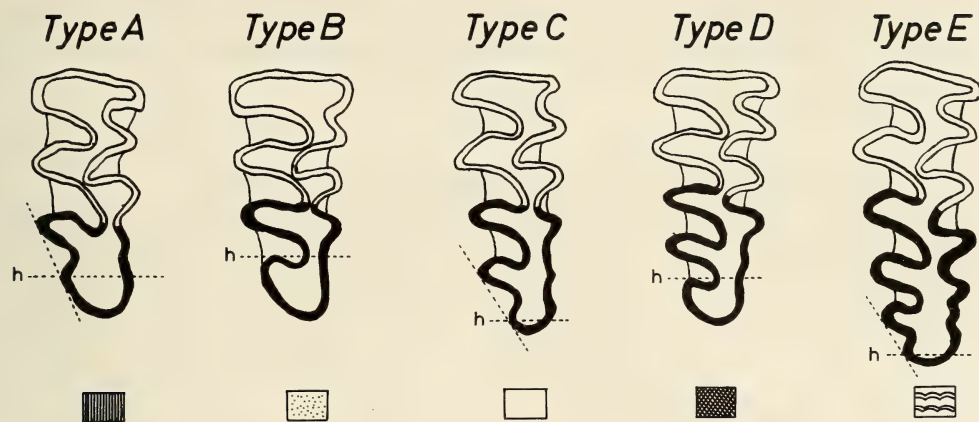
Fig. 2. Types A–E enamel patterns on the third upper molar. These patterns differ in the number of re-entrant angles and the shape of the posterior loop. The line (h), crossing at a right angle to the longitudinal line of the tooth at the antero-external margin of the last re-entrant angle, shows whether the concavity of the re-entrant angle exceeds the line posteriorly or not. Patterns of five rectangles below the molars of Types A–E are used in Figs. 4, 6, 8, 9 and 11.
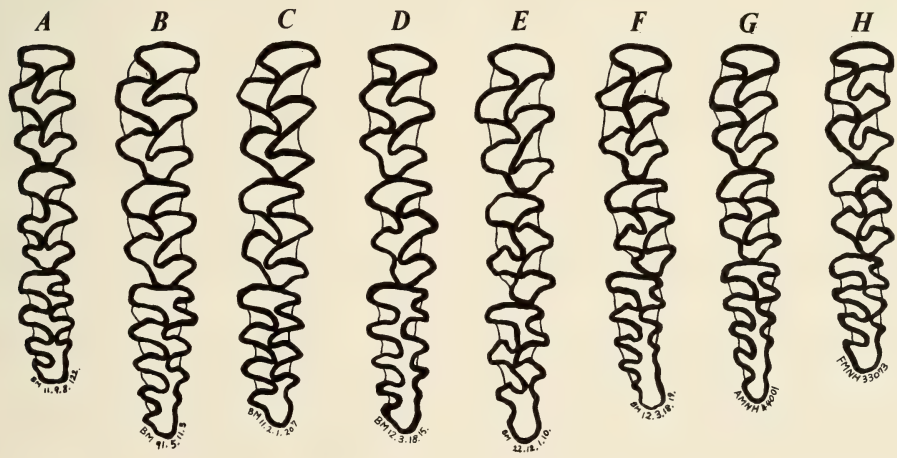
Fig. 3. Enamel patterns on the third upper molar of the *Eothenomys* holotypes examined in this study. A=MEo=*Microtus* (*Eothenomys*) *olitor* Thomas, 1911 (BM 11. 9. 8. 122), B=Mc= *Microtus chinensis* Thomas, 1891 (BM 91. 5. 11. 3), C=MAct=*Microtus* (*Anteliomys*) *chinensis tarquinius* Thomas, 1912 (BM 11. 2. 1. 207), D=MAw=*Microtus* (*Anteliomys*) *wardi* Thomas, 1912 (BM 12. 3. 18. 15), E=MAc=*Microtus* (*Anteliomys*) *custos* Thomas, 1912 (BM 12. 3. 18. 19), F=MAcr=*Microtus* (*Anteliomys*) *custos rubellus* Allen, 1924 (AMNH 44001), G=EAch= *Eothenomys* (*Anteliomys*) *custos hintoni* Osgood, 1932 (FMNH 33073), H=Ep=*Eothenomys proditor* Hinton, 1926 (BM 22. 12. 1. 10).

of the enamel lamellae of the fourth re-entrant fold protrudes beyond line "h"
(compared with Type B) ; and Type E has five salient and four re-entrant folds
with a posterior loop where the inner enamel lamellae appear as in Type A.

RESULTS

1. Variation among specimens from Sichuan and Yunnan

Thomas (1911b, 1912a) described *Eothenomys olitor* having a prominent
inner salient angle on the second upper molar, and lacking supplementary
postero-internal salient projection on the first upper molar (Fig. 3-A). Six
specimens, collected from Area V (Localities 33, 41 and 42), were identified as
E. olitor, with a TL of 35 mm, a HFL of 14-18 mm, and a CBL ranging from 20.9
to 24.1 mm ($n=5$; Fig. 4). The dominant enamel pattern on the third upper
molar was of Type B (Table 1).

Except for those of *E. olitor*, all specimens examined were provisionally
identified as belonging to one of four groups, according to the relationship
between CBL and TL, and according to the geographical areas where they were
collected (see Fig. 5). Specimens for which CBL was measured could be
grouped into two clusters in each area. Some specimens for which CBL could
be estimated were also included in, or were scattered close to their respective
clusters (except for several young specimens). Adults were included in the
respective clusters in each area except for one Area III cluster, in which no
adults appeared. In Areas I and II, there were two clusters of specimens with
longer CBL and longer TL (CI-L in Area I and CII-L in Area II) and with
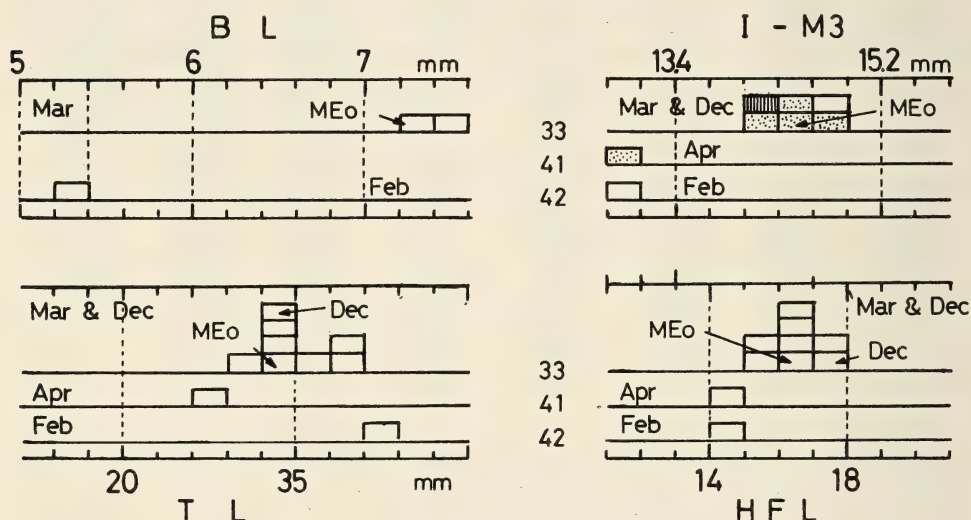
Fig. 4.   Geographical variation in BL, I-M3, TL and HFL in *Eothenomys olitor*.
One square refers to one specimen. Month indicates collecting month of specimens
examined.   For details of Localities #33, and #41-42, see the Appendix.   For enamel patterns
and abbreviation of the holotype, see Figs. 2 and 3.
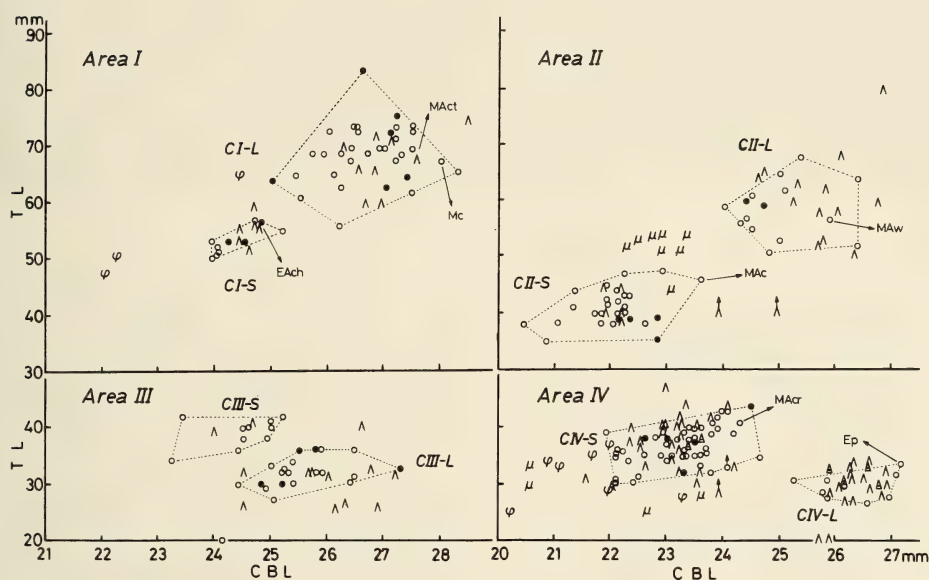
Fig. 5. Relationships between CBL and TL in Areas I–IV. For abbreviation of the holotypes (EAch, Ep, MAc, MAcr, MAct, MAw and Mc), see Fig. 3. In each area, young individuals were located to the left of a cluster of adults. Symbols: young $= \varphi$; young with estimated CBL $= \mu$; adult $= \bullet$; adult with estimated CBL $= \blacktriangle$; individual not clearly adults or young $= \circ$; individual not clearly adult or young with estimated CBL $= \wedge$; individual missing the tip of the tail $= \uparrow$. It will be shown later that clusters CI–S, CII–S, CIII–S and CIV–S correspond to *Eothenomys custos*; CIII–L and CIV–L to *E. proditor*; CI–L to *E. chinensis*; and CII–L to *E. wardi*.

shorter CBL and shorter TL (CI–S in Area I and CII–S in Area II). In Areas III and IV, there were two clusters of individulas with longer CBL and shorter TL (CIII–L in Area III and CIV–L in Area IV) and with shorter CBL and longer TL (CIII–S in Area III and CIV–S in Area IV). In each area, young individuals were found to the left of a cluster of adults: *i. e.* young in Area I=CI–L, young in Area II=CII–L, young with 20–22 mm in CBL in Area IV =CIV–S, and young with 22–24 mm in CBL in Area IV=CIV–L.

Geographical and monthly variations in two external and two skull characters (TL, HFL, BL and I–M3) along with the enamel patterns of the third upper molar, were examined for each of CI–L, CI–S, CII–L, CII–S, CIII–L, CIII–S, CIV–L, and CIV–S clusters (see Figs. 6–12). A marked difference was observed between CI–L and CII–L in the sizes of BL, TL and HFL, with only slight overlap between the two clusters in the relationship between CBL and TL (Fig. 5). TL, HFL, BL and I–M3 were slightly longer in CI–L than in CII–L and there was no clinal variation in these dimensions (see Figs. 6 and 7). In clusters

CI-L and CII-L, BL, I-M3, TL and HFL did not vary over the geographical range (Figs. 6 and 7).   Molar enamel patterns differed between clusters CI-L and CII-L (Table 1), with Type C more common in CI-L (87%) than in CII-L (68%), and Types D and E less common in CI-L (2.2%) than in CII-L (24%).

The clusters CIII-L and CIV-L overlapped (Fig. 5).   The sizes of I-M3, BL, and HFL tended to increase from north to south (Fig. 8).   Type A molar enamel was commonest in CIV-L than in CIII-L (Table 1).

Clusters CII-S, CIII-S and CIV-S all overlapped one another, but were mostly segregated from cluster CI-S (Fig. 5).   TL differed discontinuously

Fig. 6.   Geographical variation in BL and I-M3 in *Eothenomys chinensis* (CI-L) and *E. wardi* (CII-L).   One square represents one specimen.   Month indicates collecting month of specimens examined.   For detailes of Localities 1-6, 10, 12-13, and 17-21, see the Appendix.   For enamel patterns and abbreviations of the holotypes, see Figs. 2 and 3.

between clusters CI-S, CII-S and CIII-S. In cluster CII-S, I-M3 and BL decreased in size clinally from north to south, while TL and HFL did not differ among localities (Figs. 9-10). Type C molar enamel predominated in all four clusters (Table 1).

The length of I-M3 varied according to the elevation on the Lichiang Range (Locality 38), where R. C. Andrews and E. Heller (the Asiatic Expedition in 1916) and G. Forrest in 1921-22 collected specimens (Figs. 11 and 12). Both I-M3 and HFL increased in size from higher to lower elevations in cluster CIV-S, whereas they did not show a clinal change in CIV-L. Type A molar enamel
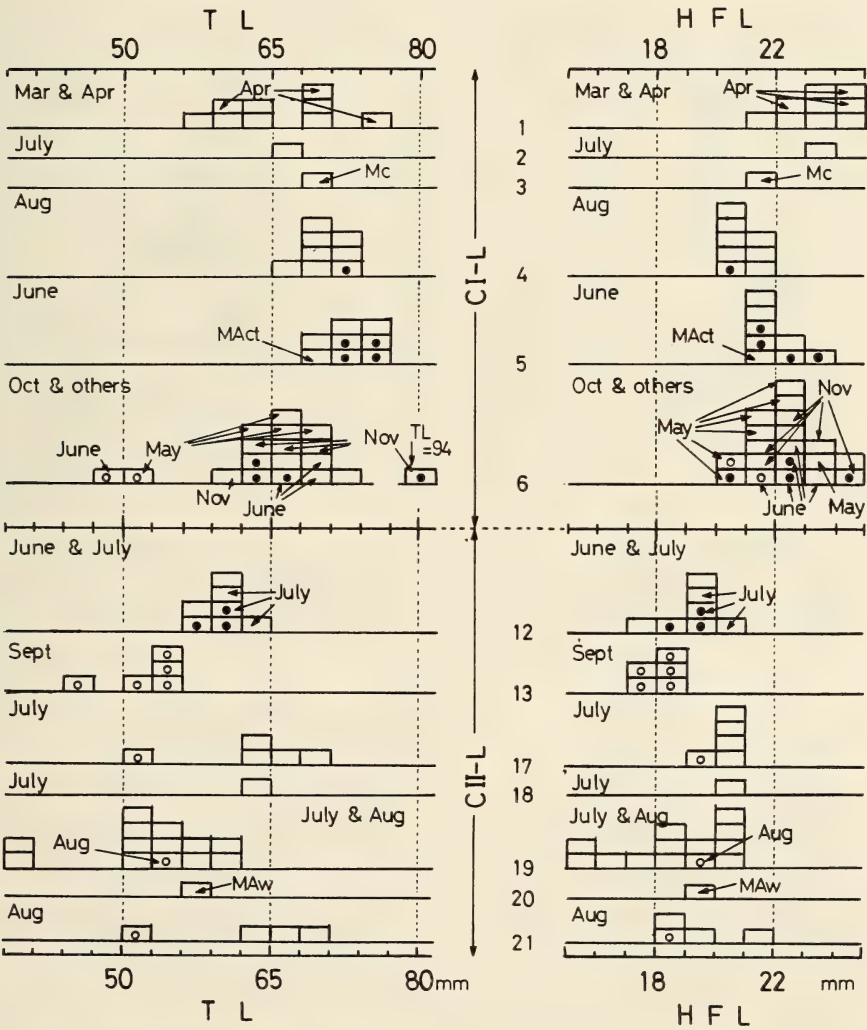
Fig. 7. Geographical variation in TL and HFL in *Eothenomys chinensis* (CI-L) and *E. wardi* (CII-L). Month indicates collecting month of specimens examined.

Table 1. Variations in the enamel patterns on the third upper molar in *Eothenomys chinensis* (ECHI), *E. wardi* (EW), *E. proditor* (EP), *E. custos* (EC), and *E. olitor* (EO).

	Type A	Type B	Type C	Type D	Type E	Total
ECHI (Area I : CI–L)	3(6.5%)	2(4.3%)	40(87.0%)	1(2.2%)	0	46
EW (Area II : CII–L)	0	3(7.3%)	28(68.3%)	8(19.5%)	2(4.9%)	41
EP (Area III : CIII–L)	13(43.3%)	11(36.7%)	5(16.7%)	1(3.3%)	0	30
EP (Area IV : CIV–L)	33(97.1%)	1(2.9%)	0	0	0	34
EC (Area I : CI–S)	0	1(5.9%)	13(76.5%)	3(17.6%)	0	17
EC (Area II : CII–S)	0	1(3.2%)	18(58.1%)	12(38.7%)	0	31
EC (Area III : CIII–S)	0	1(8.3%)	6(50.0%)	4(33.3%)	1(8.3%)	12
EC (Area IV : CIV–S)	2(2.3%)	22(25.3%)	55(63.2%)	8(9.2%)	0	87
EO (Area V)	1(12.5%)	5(62.5%)	2(25.0%)	0	0	8
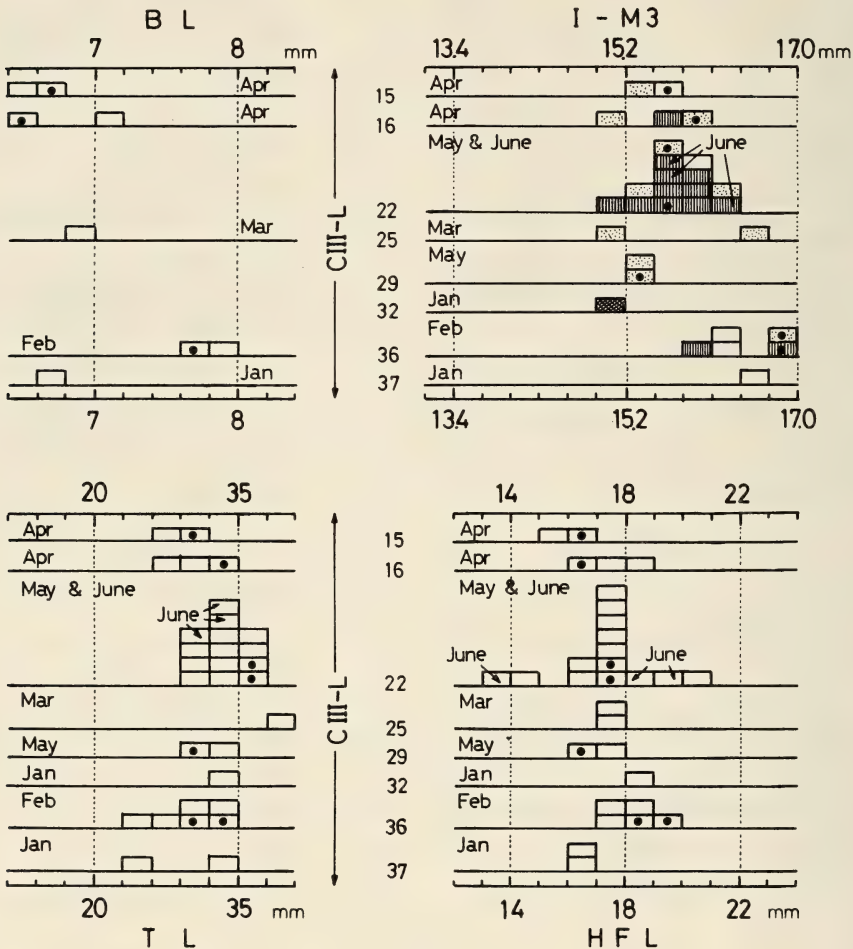
Fig. 8. Geographical variation in BL, I-M3, TL and HFL in *Eothenomys proditor* in Area III (CIII-L). Month indicates collecting month of specimens examined.
For detailes of Localities 15-16, 22, 25, 29, 32, and 36-37, see the Appendix.

was commonest in CIV–L, whereas Type C predominated in CIV–S (Table 1). Specimens from CIV–S were collected at rather higher elevations than those of CIV–L.

Adult females and young were collected in May, June, July, August and November in cluster CI-L ; in August and September in CII-L ; in February, April, and May in CIII-L ; and in May, June, and July in CI–S, CII–S and CIII–S, respectively (Figs. 6, 8 and 9). On the Lichiang Range, adult females and young were collected in August (4200 and 3300 m), September (4200 and 3900 m) and October (4500–4800, 3900 and 3600 m) in CIV–S, whereas they were captured in May (4200–3900 and 3900 m) and September (4200 and 2700 m) in CIV–L. One pregnant female collected in October in CIV–S (Locality 38,

Fig. 9. Geographical variation in BL and I-M3 in *Eothenomys custos* in Areas I–III (CI-S, CIII-S, and CII-S). Month indicates collecting month of specimens examined. For detailes of Localities 7-9, 11, 14, 22-24, 26-28, 30-31, 34-36, and 39-40, see the Appendix.

3600 m) contained two embryos (FMNH 33792).

2. Taxonomic conclusion

All 308 specimens examined in this study were found to have : i) a palatal shelf construction as in the genus *Clethrionomys* ; ii) rootless molars even in old age, and iii) narrower re-entrant folds on the molars than in the genus *Alticola* (which has little cement in the folds).   All three of these characteristics are diagnostic traits for *Eothenomys*, to which consequently they were allocated (Hinton 1926, Ellerman 1941, Corbet 1978).

Some holotypes were re-presented in the respective clusters (L and S in CI– CIV) of Areas I, II and IV (Fig. 5).   In Area I, specimens within cluster CI–
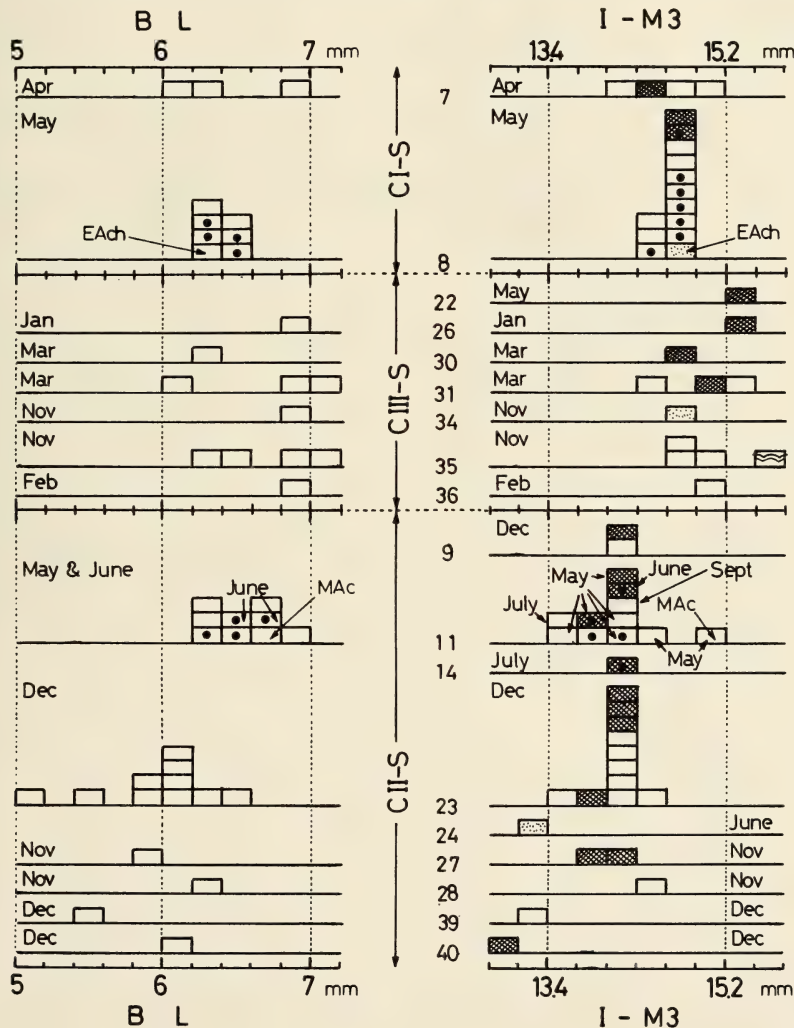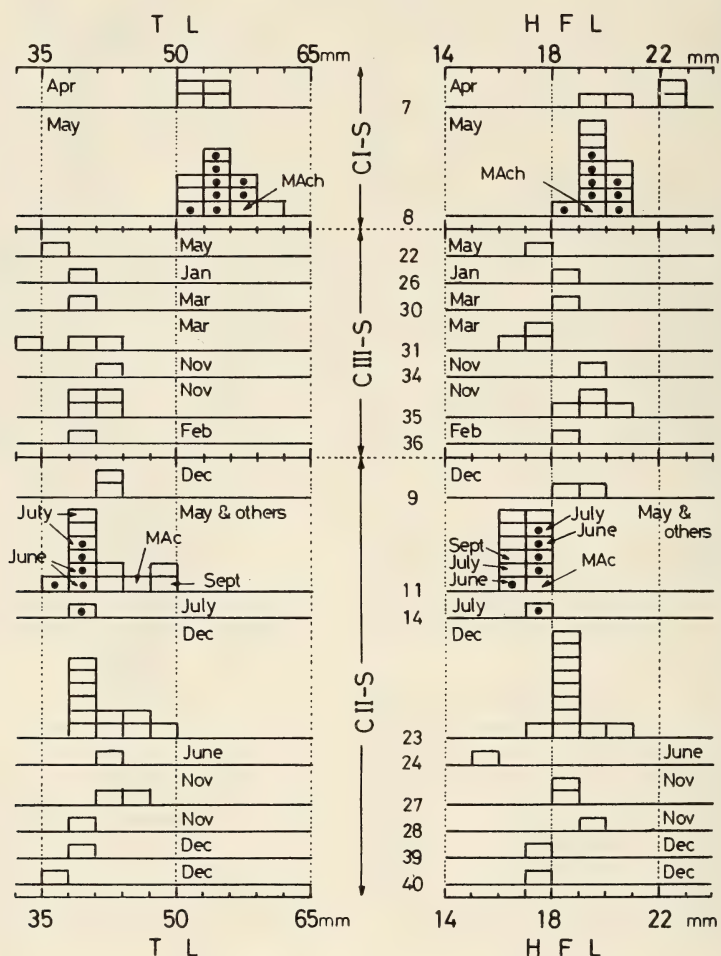

Fig. 10.   Geographical variation in TL and HFL in *Eothenomys custos* in Areas I-III (CI-S, CIII-S, and CII-S).   Month indicates collecting month of specimens examined.

L were identified as *Eothenomys chinensis* (Thomas, 1891) because the holotypes of *Microtus* (*Anteliomys*) *chinensis* Thomas, 1891 and *Microtus* (*Anteliomys*) *chinensis tarquinius* Thomas, 1912 were both included in CI-V. The latter name *Microtus* (*Anteliomys*) *chinensis tarquinius* is a junior synonym of *E. chinensis* (Thomas, 1891).

Specimens within cluster CII-L were identified as *Eothenomys wardi* (Thomas, 1912) in Area II, because the holotype of *Microtus* (*Anteliomys*) *wardi* Thomas, 1912 occurred within the cluster.
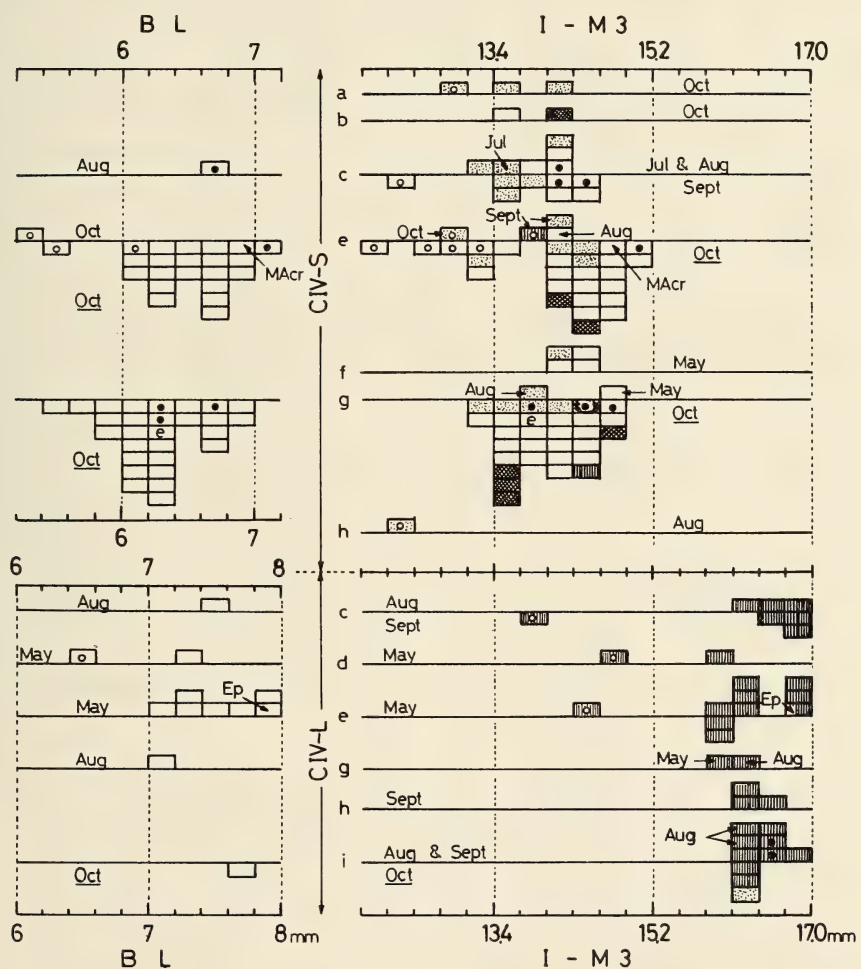
Fig. 11. Altitudinal variation in BL and I–M3 in *Eothenomys custos* (CIV–S) and *E. proditor* (CIV–L) in the Lichiang Range (locality 38). Month indicates collecting month of specimens examined. a=4500−4800 m; b=4200−4500 m; c=4200 m; d=3900−4200 m; e=3900 m; f=3600−3900 m; g=3600 m; h=3300 m; i=2700 m. Underlined records from October indicate specimens collected by R. C. Andrews and E. Heller. All other specimens were collected by G. Forrest.
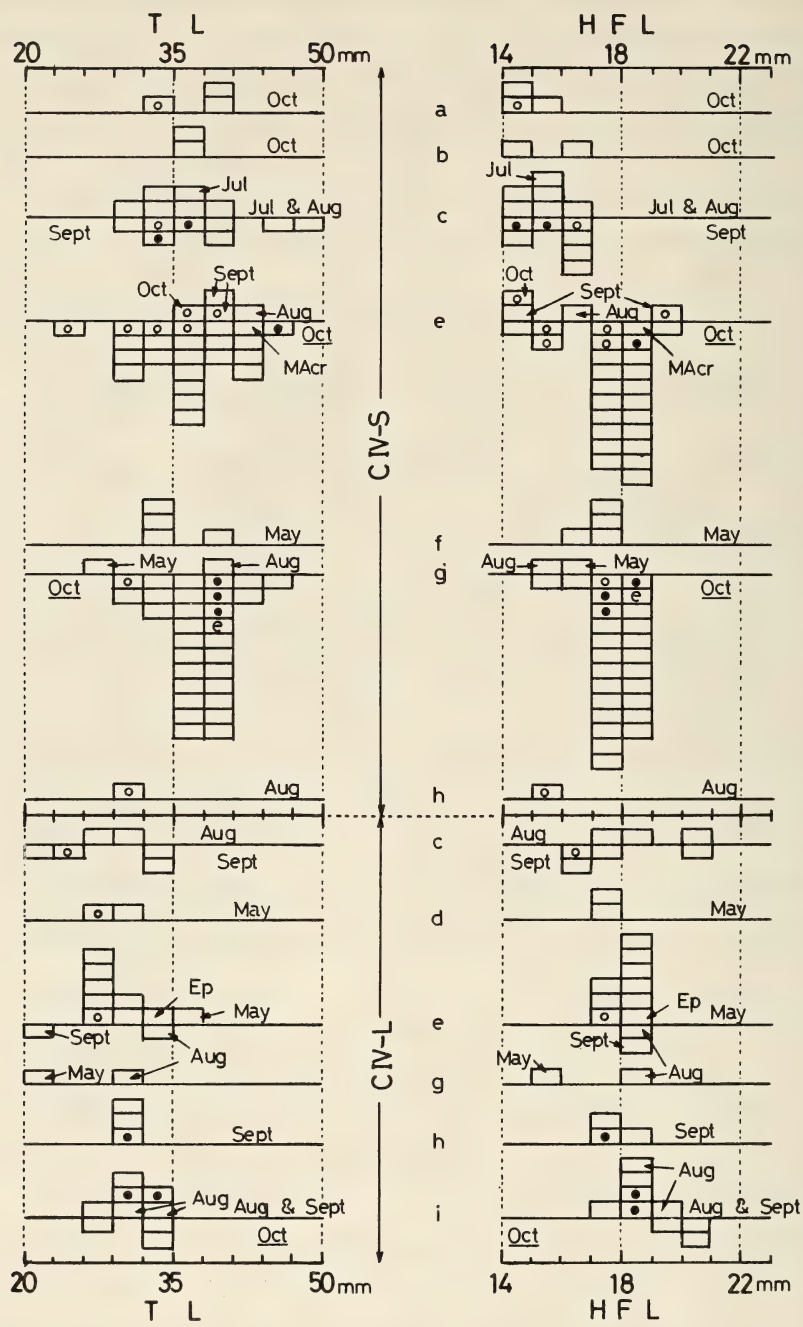
Fig. 12. Altitudinal variation in TL and HFL in *Eothenomys custos* (CIV–S) and *E. proditor* (CIV–L). Month indicates collecting month of specimens examined.

Specimens within cluster CIV-L were identified as *Eothenomys proditor* Hinton, 1923, because the holotype of *E. proditor* Hinton, 1923 was also in the cluster. In Area III, although there was no holotype, cluster CIII-L overlapped with, and was consequently regarded as conspecific with cluster CIV-L, that is *E. proditor* (Fig. 5).

Specimens within cluster CII-S were identified as *Eothenomys custos* (Thomas, 1912), because the holotype of *Microtus* (*Anteliomys*) *custos* Thomas, 1912 was included in the cluster. Clusters CIII-S and CIV-S overlapped cluster CII-S (Fig. 5); and all the specimens were identified as *Eothenomys custos* (Thomas, 1912). *Microtus* (*Anteliomys*) *custos rubellus* Allen, 1924 is a junior synonym of *E. custos* (Thomas, 1912).

It was noticeable that cluster CI-S did not overlap clusters CII-S, CIII-S or CIV-S (Fig. 5), and TL in CI-S was clearly different from those in CII-S and CIII-S (Fig. 7). However, I-M3, BL and HFL tended to either decrease or increase in size clinally, or varied continuously from north to south among these clusters. Therefore, the taxonomic position of CI-S is considered to be the same as CII-S and CIII-S, which were identified as *E. custos*. Consequently, *Eothenomys* (*Anteliomys*) *custos hintoni* Osgood, 1932, included in cluster CI-S (Fig. 5), is a junior synonym of *E. custos* (Thomas, 1912).

The relationship between H & BL and TL (tail ratio=100TL/H & BL) varied from 55-85% in *E. chinesis*, from 40-65% in *E. wardi*, from 50-65% in *E.*

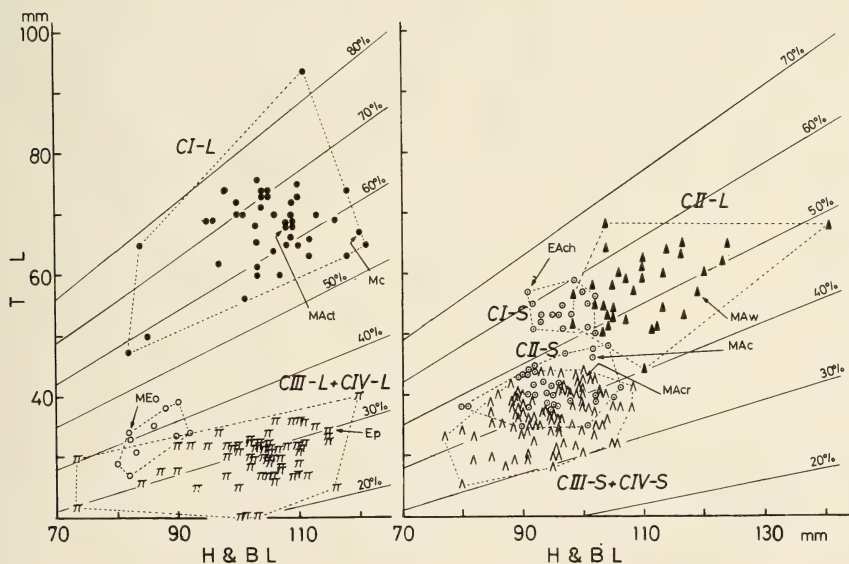Fig. 13. The relationship between H & BL and TL in five species of *Eothenomys*. The ratio of TL to H & BL is shown with lines and percentages. ● = *E. chinensis* (CI-S); ○ = *E. olitor*; π = *E. proditor* (CIII-L + CIV-L); ▲ = *E. wardi* (CII-L); ⊙ = *E. custos* in Areas I (CI-S) and II (CII-S); ∧ = *E. custos* in Areas III and IV (CIII-S + CIV-S). For abbreviations of the holotypes, see Fig. 3.

custos from Area I, and 30-50% in *E. custos* from Areas II-IV, from 30-45% in *E. olitor*, and from 20-40% in *E. proditor* (see Fig. 13).   Thus, on the basis of this character alone, it is difficult to segregate specimens of *E. chinensis* and *E. custos hintoni* from Area I, specimens of *E. custos* and *E. wardi* from Area II, or specimens of *E. custos* and *E. proditor* from Areas III and IV.

3. Latitudinal and altitudinal distributions

Eothenomys chinensis was found on both sides of the River Datu He near Omei Shan, Sichuan Province at 29-30° N.   *E. wardi* was found to occur from the Jinsha Jiang River to the Salween River around 28° N and 99° E. *E. chinensis* and *E. wardi* have allopatric ranges separated by about 240 km.   *E. custos* was found from the Yalong Jiang River to the areas between the Jinsha Jiang and Lancan Jiang (=Mekong) Rivers from 26° N to 29° N.   *E. proditor* was found along the borders of Sichuan and Yunnan Provinces, from the Yalong Jiang River to the Jinsha Jiang River around 27-28° N.   *E. olitor* was recorded from a fragmented range in Zhangton (Localities 41 and 42 ; 23° N) and Lincang (Locality 33 ; 27° N) districts in Yunnan Province.   The latitudinal distribution of *E. custos* proved to be rather larger than those of either *E. chinensis, E. wardi* or *E. proditor* (Fig. 14).

   With the exception of the fragmented range of *E. olitor*, the lower altitudinal limit of these four species of *Eothenomys* increased from north to south (see Fig. 15).   The altitudinal range of *E. chinensis*, which extends down to 1500 m, was found to be slightly lower than that of *E. wardi* which occurs above 2300 m.   *E. custos* was found at slightly higher altitudes (2500-4800 m) than *E. proditor* (2500-4200 m), though the latitudinal range of *E. custos* overlapped that of *E. proditor* in the areas of 26-28° N and 100-102° E (Fig. 14).   The lower limit of *E. custos*, range was approximately the same, at about 2500 m, in Areas I, III and IV, but in Area II it decreased from 3500 m to 2700 m from north to south.

   Some information on the habitats of *Eothenomys* spp. was available from specimen labels.   *E. wardi* was noted as occurring along the banks of streams (Locality 12), in narrow valleys in forest (Locality 18), in alpine meadows, open meadows and among rocks (Locality 13), and in alpine meadows and alpine rocks (Locality 21).   *E. custos* was noted as occurring along forested banks, in holes under trees with runs under moss (Locality 11), under roots of large trees in very damp forests (Locality 11), in alpine meadows, rocky meadows, forests, and open coniferous forests (Locality 38 ; 3300 m), and under logs (Locality 38 ; 3150 m).   *E. proditor* was found in open meadows and open rocky meadows (Locality 38), on mountain slopes (Locality 36), and under logs (Locality 37). Thus, the main habitat differences appear to be that *E. chinensis* lives in both forests and meadows, whereas *E. wardi* and *E. proditor* inhabit meadows and rock areas.
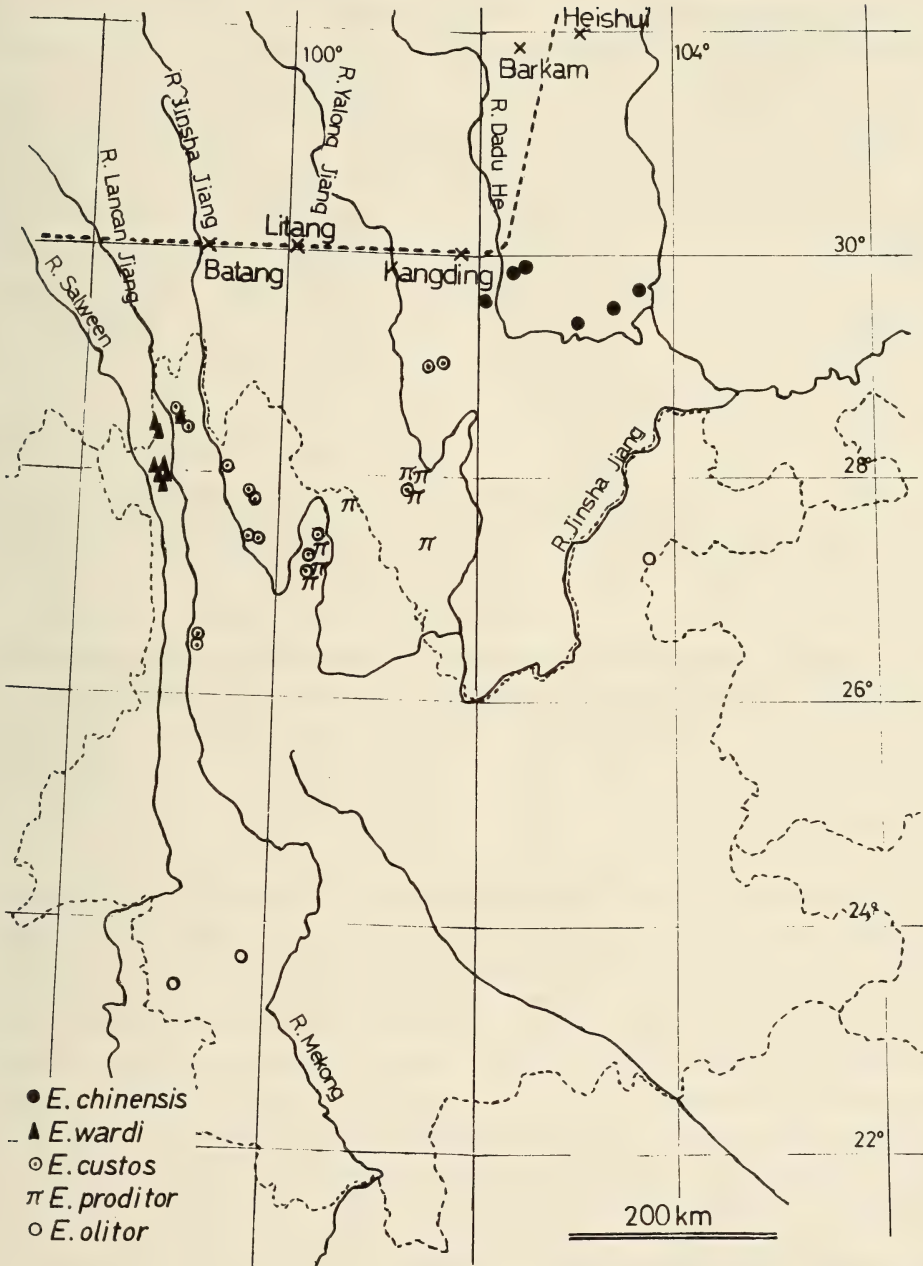
Fig. 14. Summary of the geographical distribution of *Eothenomys chinensis, E. wardi, E. proditor, E. custos,* and *E. olitor.* The broad dotted line indicates the demarcation line between the Palaearctic and Oriental regions based on mammals and birds (Zhang 1979), which passes from Zoige (33.5°N, 102.9°E), through Heishui, Barkam, Kangding and Litang, and to Batang in Sichuan Province.
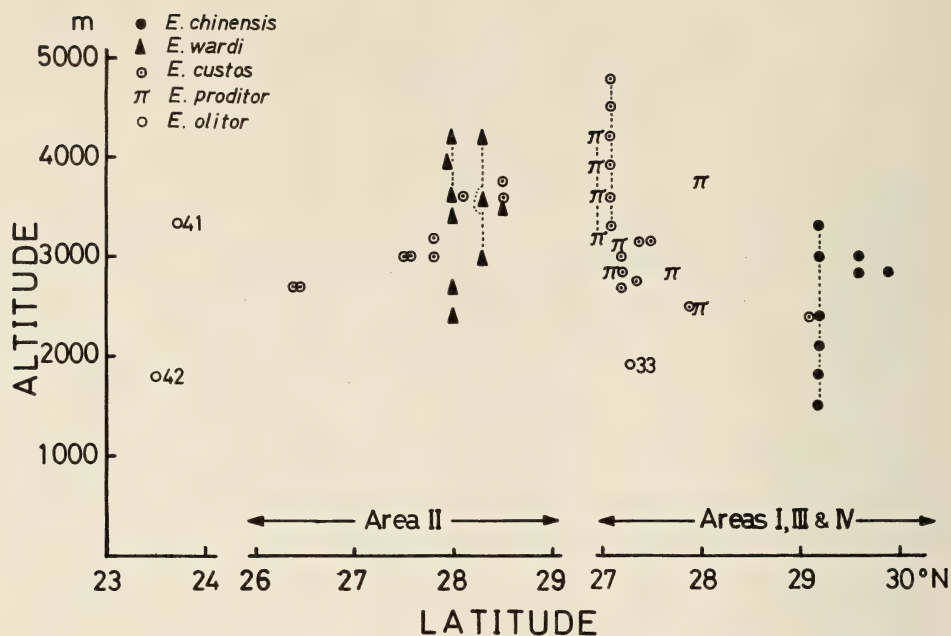
Fig. 15.   Summary of the altitudinal distribution of the five species of *Eothenomys* examined in this study.   Numbers with open circles indicates the localities of *E. olitor* listed in the Appendix.   A dotted line shows the same locality.

DISCUSSION

Hinton (1926), Ellerman (1941), and Gromov and Polyakov (1977) all considered *Anteliomys* to be a distinct genus, separate from *Eothenomys*, whereas Osgood (1932) and Allen (1940) designated *Anteliomys* as a subgenus of *Eothenomys*.   In the present study, I have followed the opinions of Ellerman (1949), Ellerman and Morrison-Scott (1951), Corbet (1978), Honacki *et al.* (1982), Corbet and Hill (1992), Musser and Carleton (1993) in regarding *Anteliomys* as a synonym of *Eothenomys*.

Two distinct groups of species belonging to the genus *Eothenomys* have been identified as occurring in the provinces of Sichuan and Yunnan.   The first is the *E. melanogaster* group, which includes *confinii, eleusis, fidelis, miletus* and *mucronatus*, and is characterized by the fourth salient angle on the first upper molar and the third salient angle on the second upper molar on the lingual side. The second group consists either of the four species *E. custos, E. chinensis, E. olitor* and *E. proditor* (Allen 1940, Ellerman and Morrison-Scott 1951, Corbet 1978, Honacki *et al.* 1982, Corbet and Hill 1991, Musser and Carleton 1993) or of the five species *E. custos, E. chinensis, E. wardi, E. olitor*, and *E. proditor* (Hinton 1926, Ellerman 1941, Gromov and Polyakov 1977, Corbet and Hill 1992),

all of which lack the inner salient angles on the first and second upper molars as found in the former group.

However, *Eothenomys* identification has remained confused due to a lack of research into morphological variation, and because only crude distribution maps have been published (Hinton 1926, Allen 1940, Corbet 1978, Corbet and Hill 1992). During research for this paper it became apparent that Allen's (1940) identification key for this species group was rather difficult to apply because of the discrepancies in the number of outer salient angles and in CBL between *E. proditor* and *E. olitor* (Figs. 3 and 5) and in TL between *E. chinensis* and *E. custos* (Fig. 13). The ratio of TL to H & BL (Hinton 1926, Corbet 1978) was not sufficient for identification because of the great overlap between the two sympatric species of *E. custos* and *E. chinensis* (or *E. wardi*) and between *E. custos* and *E. proditor* (Fig. 13). Furthermore, this study showed that the ranges of HFL and the ratio of TL to H&BL, and the number of inner re-entrant folds on the third upper molars given by Corbet and Hill (1992; Table 262) were erroneous for the five species.

The first basic taxonomic debate is over whether *wardi* is a distinct species or just a subspecies of *Eothenomys chinensis*. Thomas (1891) originally des-cribed *Microtus chinensis* from a specimen collected from Kia-ting-fu (=Le-shan; Locality 3). Later, Thomas (1911a) identified 23 specimens collected from 23 miles (=36.8km) SE of Ta-tsien-lu (=Moxi; Locality 5) and Emei Shan (Locality 4) as the same species. Subsequently, Thomas (1912b) described *Microtus (Anteliomys) wardi* from a specimen from Chamutong (=Tra-mu-tang; Kingdon Ward 1923; p. 193; Locality 20), W. of Atuntsi, Yunnan, and differentiated it from *chinensis* on the basis of its much smaller bullae. Hinton (1926) followed this classification, but Allen (1940) changed the taxonomic status of *wardi* to that of a subspecies of *chinensis*, because the third upper molar was the same as that of *chinensis*. Ellerman and Morrison-Scott (1951), Corbet (1978), Honacki *et al.* (1982), and Musser and Carleton (1993) followed Allen (1940), whereas Corbet and Hill (1992) followed Thomas (1912b) and Hinton (1926). Corbet and Hill (1992) distinguished *wardi* from *chinensis* on the basis of *wardi*'s shorter tail and smaller auditory bulla, and remarked on the length of the bulla (BL=6.7 mm in *wardi*, and 9.1 mm in *chinensis*) as a distin-guishing character. From this study, however, it is clear that in *wardi* BL ranged from 6.2 to 7.4 mm, and from 6.6 to 8.4 mm in *chinensis*. The length of 9.1 mm referred to by Corbet and Hill (1992) for *chinensis* may well be in error. I also regard *wardi* as a full species, but because not only does it have a smaller bulla but also a shorter tail and hind foot than *chinensis* (Fig. 7), and because its latitudinal distribution is isolated from that of *chinensis* (Fig. 14).

The second basic taxonomic debate is over whether *custos* is best regarded as full species or as a subspecies of *Eothenomys chinensis*. Thomas (1912b) originally described *E. (Anteliomys) custos*, based on two specimens from A-tun-tsi, Yunnan (Locality 11), which had a small bulla, and a shorter tail than either *chinensis* or *wardi*. Hinton (1926; p. 296 and p. 298–299 in the footnote), however, remarked that *custos*, was a small form very closely related mor-

phologically and geographically to the larger forms *chinensis* and *wardi*, and is best regarded as a subspecies of *Anteliomys* (=now *Eothenomys*) *chinensis*, because neither the holotype of *custos* nor the other *custos* skulls examined were "old", though Hinton (1926) retained the taxonomic position of *custos* as a full species as did Thomas (1912b) and Allen (1924). In Areas I and II, some adult females were included into clusters composed of both large (CI-L and CII-L) and small specimens (CI-S and CII-S), the last of which were clearly identified as *E. custos* (Figs. 4 and 5). Therefore, the original specimens of *custos* are neither young *chinensis* nor *wardi* as suggested by Hinton (1926).

Two subspecies of *Eothenomys custos* have been described, excluding the nominotypical subspecies. Allen (1924) described one as *Microtus* (*Anteliomys*) *custos rubellus*, collected from Ssu-shan (=Snow Mountain), in the Lichiang Range, Yunnan (Locality 38-e), on the basis that *rubellus* was a little larger on average than typical *custos*. Osgood (1932) described a second subspecies, *Eothenomys* (*Anteliomys*) *custos hintoni*, from Wushi (Wu-chi on the holotype label ; Locality 8), south-west of Tatsienlu, Sichuan, because it has a slightly longer hind foot and longer tail than *custos*. My examination showed that although the tail was longer in the specimens described by Osgood (1932), the hind foot length was not (see Fig. 10). Furthermore, Osgood (1932) stated that the interorbital width (IOW) was relatively greater in *hintoni* than in *chinensis*, and that the third inner angle of the third upper molar was usually confluent with the fourth outer one in *hintoni* but not in *chinensis*. On further examination, however, I was unable to confirm these differences : IOW ($X+SD$) of *hintoni* (Localities 7-8) was 4.38 ± 0.11 mm ($n=16$), while that of *chinensis* (Localities 1-6) was 4.33 ± 0.21 mm ($n=44$), ($t=0.784$, $0.5<p<0.6$, $df=58$). Most specimens of *chinensis* (7/9) from Locality 1, and the holotype of *chinensis tarquinius*, had enamel lamellae contacting the third inner and the fourth outer triangles, whereas other specimens of *chinensis* from Localities 2-6, and of *custos* from Localities 7-8, did not.

The range of *Eothenomys chinensis* was shown to be distinct from but parallel to that of *E. custos* in Sichuan (Allen 1940). That the two species are allopatric in distribution has been further confirmed by the present study. The distribution of *E. chinensis* is also known to be distinct from that of *E. eva* (Kaneko 1992).

Eothenomys custos has been recorded from the extreme north-west of Yunnan, the Likiang Range, the loop of the Jinsha Jiang River, and from central Sichuan (Hinton 1926, Allen 1940). Yang (1985), added Lanping (26.4° N, 99.2° E), Jianchuan (26.5° N, 99.8° E), and Dali (25.6° N, 100.1° E) to the range of *E. custos*, though his means of identification was not clear. Because *E. custos* has been recorded from around 25.5-29° N and from 99-100.5° E (Fig. 14), the latitudinal distribution is the largest among *Eothenomys* species investigated here. *E. custos* was not, however, recorded from the west side of the Lancan Jiang River (Figs. 1 and 14). According to Yang (1985), the lower limit of *E. custos*, range decreases from north to south. The present study supports his finding in Area II, but not in Areas I, III and IV, where the lower limits were

nearly the same (Fig. 15). Therefore, the altitudinal distribution of *custos* probably differs between Sichuan and Yunnan Provinces. The habitat of *custos* studied here was also similar to that reported by Yang (1985); *i. e.* it occurs in shrubs, bamboos, alpine meadows, and in forests.

No taxonomic problems have been associated with *Eothenomys proditor* since Hinton (1923) described it on the basis of its generally smaller size, its shorter tail and peculiar third upper molar (simple form) based on specimens collected from the Lichiang Range (Locality 38). Although there have been no published reports of the geographical range or habitat of this species, I consider *E. proditor* to be restricted to the border between Sichuan and Yunnan, at around 27–28° N and 100–102° E, and that it lives in meadows and in rocky areas.

Eothenomys olitor, the least abundant member of the genus, was described as a new species, *Microtus (Eothenomys) olitor* by Thomas (1911b) on the basis of specimens collected at Chao-tung-fu (Locality 33), in eastern Yunnan. *E. olitor* differs considerably from other forms examined here. On the second upper molar, although a third inner salient angle appears in some specimens of *E. custos, E. proditor, E. chinensis* and *E. wardi* as a very small form, the salient angle is as large in *E. olitor* as any species of the *E. melanogaster* group (Fig. 3).

The range of *Eothenomys olitor* has been recorded as fragmentary and widely scattered (Fig. 14). Allen (1924) recorded Mucheng, Salween Drainage (2100 m; Locality 42) as a new locality for the species. Later, Allen (1940; p. 820) reported one specimen of *E. olitor* collected from Peitai, 30 miles (=45km) south of Chungtien, near Locality 27, from within the range of *E. custos* (Fig. 14). I was not able to locate the specimen in the museums examined here, because Allen (1940) did not record the registration number of the specimen. Two specimens, housed in MCZ and AMNH, belonging to the *E. melanogaster* group, were, however, collected at Petai on November 26, 1916, by R. C. Andrews. One of them (now MCZ 21298 and originally AMNH 44109) had two re-entrant angles on the lingual side of the third upper molar and the other (AMNH 44233) had three. I think that Allen (1940) misidentified these specimens as *E. olitor*. The latitudinal distribution, therefore, does not include western Yunnan, as described by Allen (1940), but north-eastern and south-western Yunnan, and the altitudinal ranges extends from 1800 to 3350 m. The habitats *E. olitor* occurs in were noted as cultivated plains (Locality 33; Thomas 1912a) and rhododendron shrubs on Daxue Shan (Locality 41; Lu *et al*. 1965).

The altitudinal range of the four species of *Eothenomys* here tended to increase from north to south (Fig. 15). The lower limits of their altitudinal distribution does not, however, coincide with the distribution of vegetation types on the mountains of Sichuan and Yunnan Provinces (Xibei Teachers College and Ditu Chubanshe 1984, Yunnan Province Epidemiology Institution 1978), except for *E. olitor* due to its very fragmented distribution. As an example, *E. custos* was recorded from 1500 to 3300 m on Emei Shan. There, evergreen and deciduous mixed forests occurs from 1500 to 2000 m, coniferous

and broad-leaved mixed forests from 2000 to 2800 m, and subalpine, shrubby, meadowy and coniferous zones above 2800 m (Xibei Teachers College and Ditu Chubanshe 1984).   As a second example, *E. wardi* occurs from 2400 to 4200 m around 28° N, however, vegetation in the region changes from *Pinus yunnanensis* and *P. armandii* which grow from 2500 to 3000 m, to mixed forests with *P. yunnanensis, Betula* spp. and *Quercus* spp. from 3000 to 3500 m, various *Picea* spp. from 3500 to 4000 m, and to alpine shrubs and meadows with *Rhododendron* spp. above 4000 m (Yunnan Province Epidemiology Institution 1978).   It seems likely that the distributions of the four *Eothenomys* species are affected more by topographical barriers, such as rivers running along Hengduan Shan, than by vegetation type.

The length of I–M3 in *Eothenomys custos* increased from south to north (Fig. 9), conversely that of *E. proditor* increased from north to south (Fig. 8). Given the significant correlation between I–M3 and CBL, it also means that body size increases or decreases from south to north, that is an example of Bergmann's rule or of reverse Bergmann's rule.   Many mammalian species ranging through wide latitudes follows the rule or the reverse of Bergmann's rule (McNab 1971), thus some species of *Microtus* (Arvicolidae) living in northern latitudes above 50° N obey Bergmann's rule, whereas those living in southern latitudes below 50° N obey the reverse of the rule (Kaneko 1988).   It is particularly interesting that two opposite clines in skull length are to be found in closely adjacent areas of Sichuan and Yunnan Provinces.

The breeding seasons of the various voles could be estimated by the occurrence of young and adult females with developed mammae (Figs. 6, 9 and 11).   The breeding seasons of *E. chinensis, E. wardi*, and *E. custos* were mainly from early summer to late fall, whereas that of *E. proditor* was from February to May in Area III and from spring to fall in Area IV.   Thus, *E. proditor* probably breeds slightly earlier than do other species of *Eothenomys*.

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APPENDIX

1. Lu Tsing Shan (=Luding Xian), Sichuan; 29.9° N, 102.3° E; chinensis (March, 1931; FMNH 36527−29, 36531−32, 36536, 36538/ April 1931; FMNH 36534−35, 36537).
2. Erlang Shan, Sichuan; 29.9° N, 102.2° E; 2880 m; chinensis (July 1962; ASZI 20831).
3. Kia-ting-fu (=Leshan), Sichuan; 29.6° N, 103.7° E; the date of collection remains unknown; BM 91.5.11.3 (the holotype of Microtus chinensis Thomas, 1891).
4. Omi-san (=Emei Shan), Sichuan; 29.6° N, 103.4° E; 2850 m; chinensis (August 1910; BM 11.2. 215−222).
5. 23 miles SE of Ta-tsien-lu (=Moxi), Sichuan; 29.6° N, 102.1° E; 3000 m; June 1910; BM 11.2.1. 207 (the holotype of Microtus (Anteliomys) chinensis tarquinius Thomas, 1912); chinensis (June 1910; BM 11.2.1.208−214).
6. Washan, Sichuan; 29.2° N, 103.0° E; 1500 m, 1800 m, 2100 m, 2400 m, 3000 m, 3300 m; chinensis (May 1908, MCZ 7815, 7817, 7819, 7821−23, 7825/ June 1908; MCZ 7812−14, 7820, 7824/ October 1908; MCZ 7805, USNM 175141/ November 1908; MCZ 7806−7809, BM 13.9.13.9/ July 1925; USNM 241279, 241282).
7. Tong Ku or Chung Ku, Chu Liang Shiang (=Jiulong Xian), Sichuan; 29.1° N, 101.6° E; 2400 m; custos (April 1934; AMNH 113555−56, 113559−60).
8. Wu-chi (=Wuxu), SW of Tatsienlu, Sichuan; 29.1° N, 101.4° E; May 1929; FMNH 33073 (the holotype of Eothenomys (Anteliomys) custos hintoni Osgood, 1932); custos (May 1929; FMNH 33072, 33075−76, 33079−80, 33083−33085, 33218; BM 1938.4.1.184−185; USNM 259917−18).
9. Adong, Yunnan; 28.7° N, 98.5° E; custos (December, 1979; ASZI 79806−07).
10. E of Atuntzi (=Deqen Xian), Yunnan; 28.5° N, 98.9° E; 3588 m; wardi (the date of collection remains unknown; BM 22.10.21.8, 22.10.21.11−13, 22.10.21.15).
11. A-tun-tsi, (=Deqen Xian), Yunnan; 28.5° N, 98.9° E; 3600−3750 m; May 1911; BM 12.3.18.19

(the holotype of *Microtus* (*Anteliomys*) *custos* Thomas, 1912) ; *custos* (May 1911 ; BM 12.3.18.16—18, 12.3.18.21—23/ June 1911 ; BM 12.3.18.24, 14.10.23.31/ September 1911 ; BM 12.3.18.20/ 3560 m ; July 1960 ; ASZI 17115).

12. Doker-la, Yunnan ; 28.3° N, 98.7° E ; 3600 m ; *wardi* (June 1913 ; BM 14.10.23—25, 14.10.23.28/ July 1913 ; BM 14.10.23.26—27, 14.13.23.29—30).

13. Mekong-Salween Divide (=near Dokerla), Lat. 28° 20 N, Yunnan ; 28.3° N, 98.7° E ; 3000—4200 m ; *wardi* (September 1921 ; BM 22.12.1.27, 65.3836, 65.3839—41).

14. Benzilan, Yunnan ; 28.1° N, 99.3° E ; 3600 m ; *custos* (July 1960 ; ASZI 17186).

15. Kulu (=I-tse), Szechwan ; 28.0° N, 101.3° E ; *proditor* (April 1929 ; FMNH 33064, 33070).

16. I-tze Camp, Szechwan ; 28.0° N, 101.3° E ; 3750 m ; *proditor* (April 1929 ; FMNH 33067—69).

17. Mekong Valley (=near Tzeka), Lat. 28° N, Yunnan ; 28.0° N, 98.9° E ; 2400—2700 m ; *wardi* (July 1921 ; BM 22.12.1.18—20, 65.3832, 66.1998).

18. SW side of Si-la pass, Yunnan ; 28.0° N, 98.7° E ; 3420 m ; *wardi* (July 1922 ; BM 22.10.21.6).

19. Mekong-Salween Divide, Lat. 28° N, Yunnan ; 28.0° N, 98.7° E ; 3600-4200 m ; *wardi* (July 1921 ; BM 22.12.1.21—26, 22.12.1.28—30, 65.3834—35, 65.3842/ August 1921 ; BM 65.3837).

20. Chamutong (=Tra-mu-tang ; Kingdon Ward, 1923), Upper Salween drainage-area, W of A-tuntsi, Yunnan ; 28.0° N, 98.6° E ; 3900 m ; the date of collection remains unknown ; BM 12.3.18.15 (the holotype of *Microtus* (*Anteliomys*) *wardi* Thomas, 1912).

21. Kiu-Chiang-Salween Divide (=near Gompa La), Lat. 28° N, Yunnan ; 28.0° N, 98.5° E ; 3600—4200 m ; *wardi* (August 1921 ; BM 22.12.1.31—33, 65.3838).

22. Muli, Szechwan ; 27.9° N, 101.3° E ; 2500 m ; *custos* (May 1959 ; ASZI 17426) ; *proditor* (May 1959 ; ASZI 17421, 17425, 17428, 17430—31, 17434, 17439/ May 1960 ; ASZI 17422, 17424, 17427, 17433/ June 1960 ; ASZI 17423, 17437, 17440).

23. To-mu-lang, Chung-tien Dist. (=near Zhongdian Xian), Yunnan ; 27.8° N, 99.7° E ; 3000 m ; *custos* (December 1916 ; AMNH 44201, 44203—04, 44209—10, MCZ 21303—05, FMNH 33934—36).

24. Zhongtian Xian, Yunnan ; 27.8° N, 99.7° E ; 3200 m ; *custos* (June 1959 ; ASZI 17183).

25. Yun-ning (=Yongning), Yunnan ; 27.7° N, 100.8° E ; 2850 m ; *proditor* (March 1929 ; FMNH 33019, 33060).

26. Chang Sung Ping, 60 miles N Lichiang, Yunnan ; 27.5° N, 100.4° E ; 3150 m ; *custos* (January 1929 ; FMNH 32540).

27. 20 miles S Chungtien, Tugan-sha, Yunnan ; 27.5° N, 99.7° E ; 3000 m ; *custos* (November 1911 ; FMNH 33937—38).

28. Pesu Rusi (=near Xiazhongdian), Lichiang, Yunnan ; 27.5° N, 99.7° E ; 3000 m ; *custos* (November 1916 ; FMNH 33797).

29. Yannyan, Szechwan ; 27.4° N, 101.5° E ; *proditor* (May 1959 ; ASZI 17420, 17432).

30. Big Bena, Lichiang Range, Yunnan ; 27.4° N, 100.4° E ; 3180 m ; *custos* (March 1929 ; FMNH 33015).

31. Lutzulu, Lichiang Range, Yunnan ; 27.4° N, 100.4° E ; 2790 m ; *custos* (March 1929 ; FMNH 33012—14).

32. 45 miles N Lichiang, Yunnan ; 27.4° N, 100.4° E ; *proditor* (January 1929 ; FMNH 32539).

33. Chao-tung-fu (=Zhaotong Xian), Yunnan ; 27.3° N, 103.7° E ; March 1911 ; 1920 m ; BM 11.9.8.122 (the holotype of *Microtus* (*Eothenomys*) *olitor* Thomas, 1911) ; *olitor* (March 1911 ; BM 11.9.8.121, 11.9.8.123—24, 11.9.8.126/ December 1963 ; ASKZI 631407).

34. Peh-hsui (=near Daju), Lichiang, Yunnan ; 27.2° N, 100.4° E ; 3000 m ; *custos* (November 1916 ; AMNH 44018).

35. Taku Hills (=near Daju), Lichiang, Yangtze River, Yunnan ; 27.2° N, 100.4° E ; 2700 m ; *custos* (November 1916 ; FMNH 33798—800, MCZ 21310).

36. Nguluko, Yunnan ; 27.2° N, 100.3° E ; 2850 m ; *custos* (February 1929 ; FMNH 33009) ; *proditor* (February 1929 ; FMNH 33003—04, 33006—07, 33010, USNM 259908).

37. 25 miles N Lichiang, Yunnan ; 27.2° N, 100.3° E ; 3150 m ; *proditor* (January 1929 ; FMNH 32537—38).

38. Lichiang Range (=Yulongxuen), Yunnan ; 27.1° N, 100.2° E.

a. 4500—4800 m ; *custos* (October 1922 ; BM 75.645—46, FMNH 28964).

b. 4200—4500 m ; *custos* (October 1922 ; BM 75.651, FMNH 28963).

c . 4200 m ; *custos* (July 1922 ; BM 23.10.11.7/ August 1922 ; BM 23.10.11.4−5, 75.652−53, 75.655/ September 1922 ; BM 23.10.11.12, 75.647−49, 75.654, 76.656−58) ; *proditor* (August 1922 ; BM 75. 682, 75.684−85, FMNH 28967/ September 1922 ; BM 75.682−683, 75.686−687).

d . 3900−4200 m ; *proditor* (May 1921 ; BM 22.12.1.11, 22.12.1.12).

e . 3900 m ; October 1916 ; AMNH 44001 (the holotype of *Microtus* (*Anteliomys*) *custos rubellus* Allen, 1924) ; *custos* (October 1916 ; AMNH 44003, 44005, 44116−18, 44120, 44123, 44126−28, 44131−33, 44135−36, MCZ 21309, 21311−12, FMNH 31693, 33783, 33784 (the skin is now housed in the AMNH as 44119), 33785−86, 33788, USNM 259928−29/ August 1922 ; BM 75.662/ September 1922 ; BM 75.664, 75.678/ October 1922 ; BM 75.665) ; May 1921 ; BM 22.12.1.10 (the holotype of *Eothenomys proditor* Hinton, 1923) ; *proditor* (May 1921 ; BM 22.12.1.13−14, 22.12.1.16−17, 65. 3828−30, 75.675/ August 1922 ; BM 75.676/ September 1922 ; BM 75.681).

f . 3600−3900 m ; *custos* (May 1921 ; BM 22.12.1.15, 65.3825−26, 75.661).

g . 3600 m ; *custos* (October 1916 ; AMNH 44007, 44010−11, 44140−43, 44147, 44149−50, 44152, 44154−56, 44158−60, 44163, 44168, FMNH 33789, 33792−96, MCZ 21306−08, USNM 259930, BM 23.3.17.112/ May 1921 ; BM 75.660/ August 1922 ; BM 75.663) ; *proditor* (May 1921 ; BM 65.3824/ August 1922 ; BM 23.10.11.2).

h . 3300 m ; *custos* (August 1922 ; BM 75.666) ; *proditor* (September 1922 ; BM 23.10.11.9, 23.10.11.11, 75.671).

i . 2700 m ; *proditor* (October 1916 ; AMNH 44015, FMNH 31691, MCZ 21293/ August 1922 ; BM 23. 10.11.3, 75.669, 75.674/ September 1922 ; BM 23.10.11.6, 75.670, 75.674, FMNH 28968−69).

39 . La-chu-mi (=near Langpig Xian), Mekong River, Yunnan ; 26.4° N, 99.2° E ; 2700 m ; *custos* (December 1916 ; MCZ 21302).

40 . Ying-pan-kai (=Yingpan), Mekong River, Yunnan ; 26.4° N, 99.1° E ; 2700 m ; *custos* (December 1916 ; AMNH 44037).

41 . Daxue Sahn, Yongde, Yunnan ; 23.7° N, 99.7° E ; 3350 m ; *olitor* (April 1964 ; ASZI 23960).

42 . Mucheng, Salween Drainage (=Megdingjie), Yunnan ; 23.5° N, 99.1° E ; 1800 m ; *olitor* (February 1917 ; MCZ 21285).

On the specific names of the Japanese moles of the genus *Mogera* (Insectivora, Talpidae)

Masaharu MOTOKAWA and Hisashi ABE[1]

Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606–01, Japan ;
[1]*Laboratory of Agro-forest Ecology, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*
Fax. 075-753-4114, e-mail. masaharu @ zoo. zool. kyoto-u. ac. jp

Abstract. The original designation of the lectotype of *Mogera wogura* (Temminck, 1842) by Corbet (1978) is incomplete, but the specimen RNH28684 which Corbet probably intended to designate is taken as the lectotype. Moles from the southern half of the Japanese main islands well coincide with RNH28684 in important diagnostic characters. Thus the name *M. wogura* should be given to these moles as concluded in Abe (1995). The name *M. minor* Kuroda, 1936 which Abe (1995) adopted for the moles found in the northern half is invalid and should be changed to *M. imaizumii* (Kuroda, 1957) in accordance with the Article 59b of the International Code of Zoological Nomenclature, the third edition (1985). Except for the name alternation from *M. minor* to *M. imaizumii*, there is nothing to change the synonym list for this species and for *M. wogura* in Abe (1995).

Key words : *Mogera imaizumii, Mogera minor, Mogera wogura*, taxonomic revision.

Three species of *Mogera* Pomel, 1848 occur in Japan : *M. wogura* (Temminck, 1842) occupying the southern part of the main islands, *M. minor* Kuroda, 1936 (= *M. imaizumii* (Kuroda, 1957) as revised in this paper) found in the northern part except Hokkaido, and *M. tokudae* Kuroda, 1940 restricted to Sado Island and a part of Echigo Plain in northwestern Honshu (Abe 1995). In the taxonomic revision of *Mogera*, Abe (1995) employed the type series of specimens as the type of *M. wogura*, without comments on the lectotype which was inadequately designated by Corbet (1978). This procedure, however, is not sufficient and remains some vagueness. One of us (HA), consequently, re-examined in detail some of the important diagnostic characters of the type series. One of the purposes of this paper is to give the result of the examination. *M. minor* which is adopted in Abe (1995) for the northern species is not correct on reference to the International Code of Zoological Nomenclature (ICZN), the third edition (1985), so that revising of the name is the other purpose of this paper. Another species, *M. tokudae* is excluded from the present discussion, because of the very different characters from those of the type series and also from the above two species (Abe 1995).

MATERIALS AND METHODS

The type series of *Talpa wogura* (=*M. wogura*) from "Japan" without specified localities (Temminck 1842) in the Rijksmuseum van Natuurlijke Historie (RNH) in Leiden and the British Museum (Natural History)(now Natural History Museum) (BM) in London, and all the other specimens of Japanese moles used by Abe (1995) were examined. In addition to the above, twelve specimens housed in the Hokkaido University (HU) and the National Science Museum, Tokyo (NSMT) were also examined : four specimens (HU, A5846-5849) from Sano, Tochigi Prefecture ; five specimens (NSMT M1637, 7597, 8510, 9424, 15808) from Tokyo ; two specimens (NSMT M1633, 1639) from Tochigi Prefecture ; and one specimen (NSMT M11890) from Ushikunuma, Ibaraki Prefecture.

The southern species (*M. wogura*) is generally larger than the northern one (*M. imaizumii*), but the size is geographically variable (Abe 1967). The most effective diagnostic characters for them are ; 1) the difference between the length of upper tooth row (I^1-M^3) and the length from the front margin of upper canine to the rear margin of the third upper molar (C-M^3), 2) the degree of projection of upper incisor row, calculated as percentage of the difference between I^1-M^3 and C-M^3 to the rostral width at canines (data were arcsin-root transformed to compare with those of Abe 1995), and 3) the shape of the upper incisor row. The southern species has a round arc-like upper incisor row, a smaller difference between I^1-M^3 and C-M^3, and a smaller degree of projection of the upper incisor row, in contrast to a V-shaped upper incisor row, a larger difference between I^1-M^3 and C-M^3, and a larger degree of projection of the upper incisor row in the northern species (Abe 1967, 1995).

Since these diagnostic characters become less effective with advancing age (Abe 1967, 1995), all the skull specimens examined were assessed as belonging to one of four age classes (=ac I-IV) following the methods of Hoslett and Imaizumi (1966) and Abe (1967), and the specimens of ac III and IV were not used in the graphical comparison. The greatest length of skull (GL in mm) was used as the size character to examine graphycally the relationship with the difference between I^1-M^3 and C-M^3, and with the degree of projection of upper incisor row.

For the broken skulls in the type series of which GL could not be measured, their GLs were estimated from the following regression formulas with the length of mandibles (LM in mm) of 32 specimens of *M. wogura* (ac I and II) from Kyushu and 32 specimens of *M. imaizumii* (ac I and II) from Nagano and Miyagi Prefectures, Honshu : A, for *M. wogura*, GL=8.598+1.200LM (r^2= 0.860) and B, for *M. imaizumii*, GL=9.048+1.167LM (r^2=0.942). In these two regression formulas, there are no significant differences between the regression coefficients (ANCOVA : p=0.076) and also between the variances (ANCOVA : p=0.847).

Of the 17 specimens carrying skulls in the type series, RNH16244 (ac II),

RNH16249 (ac I, published as "lectotype" by Corbet 1978), RNH16250 (ac I), and RNH28696 (ac I) retain complete skulls, while skulls of RNH16245 (ac I), RNH28682 (ac I), RNH28684 (ac II, intended as lectotype by Corbet 1978, see Discussion), RNH28694 (ac II), RNH28695 (ac I), and RNH28699 (ac II) are incomplete and their GLs are estimated by the formula A or by the two formulas (A and B). Four aged specimens (ac III and IV, RNH16246–8 and RNH28698) and three incomplete specimens (ac I, RNH28697 carrying only mandible, RNH28700 carrying a broken skull but lacking mandible, and BM43 12 27 5 carrying a broken skull with mandible) were not used in the graphycal comparison.

RESULTS

As indicated in Figs. 1 and 2, RNH16249 which was designated as the "lectotype" of *M. wogura* by Corbet (1978) and four paralectotypes (RNH16245, 16250, 28695, 28696) in age class I well coincides with small forms of the southern mole in Tanegashima Island, Yakushima Island, Tsushima Island and a part of Kyushu, although one of the four paralectotypes (RNH16250) is somewhat marginal in position. The shape of the upper incisor row in RNH16249 and the four paralectotypes is typically round arc-like, and well corresponds to that of the southern species.

In Figs. 1 and 2, parts of Shiojiri (e) and Nakano (f) populations in the northern species overlap with Yakushima (C) population and the smaller form of Kyushu (D) population in the southern species. However, the geographical range of these northern mole populations is far from that of the southern mole populations, and in case that the two species occur proximately, the size is very different such as between the Shiojiri population (e) of the northern species and the Ina population (G) of the southern one, and between the northern one in Agematsu (d) and the very large southern one in Kiso (I).

One paralectotype (RNH28682), the largest specimen in the type series of skulls, on the other hand, is clearly excluded from the group of the southern species and is close to the larger form of the northern species such as of Kanto Plain (g), Sendai Plain (Semine, h) or the larger individuals of northwestern Honshu (c) (Figs. 1 and 2). The upper incisor row of RNH28682, however, is rather deep arc-like as found in young and small individuals of the southern species.

In Figs. 3 and 4, RNH28684 which Corbet (1978) probably intended to designate as the lectotype of *M. wogura* (see Discussion) is marginal for both of the species, *i. e.* this specimen is included in the range of Nakano (f) population of the northern species or very close to the population of Yakushima Island (C) and to the smaller individuals of Kyushu (D) and Hiroshima (E) populations. The shape of the upper incisor row, however, is clearly round arc-like, and is the same as that of RNH16249 and as that of the southern species, although the arc is slightly shallower in RNH28684 due to the advanced age (ac II). Thus, RNH28684 is likely a specimen of the southern species, but not of the northern
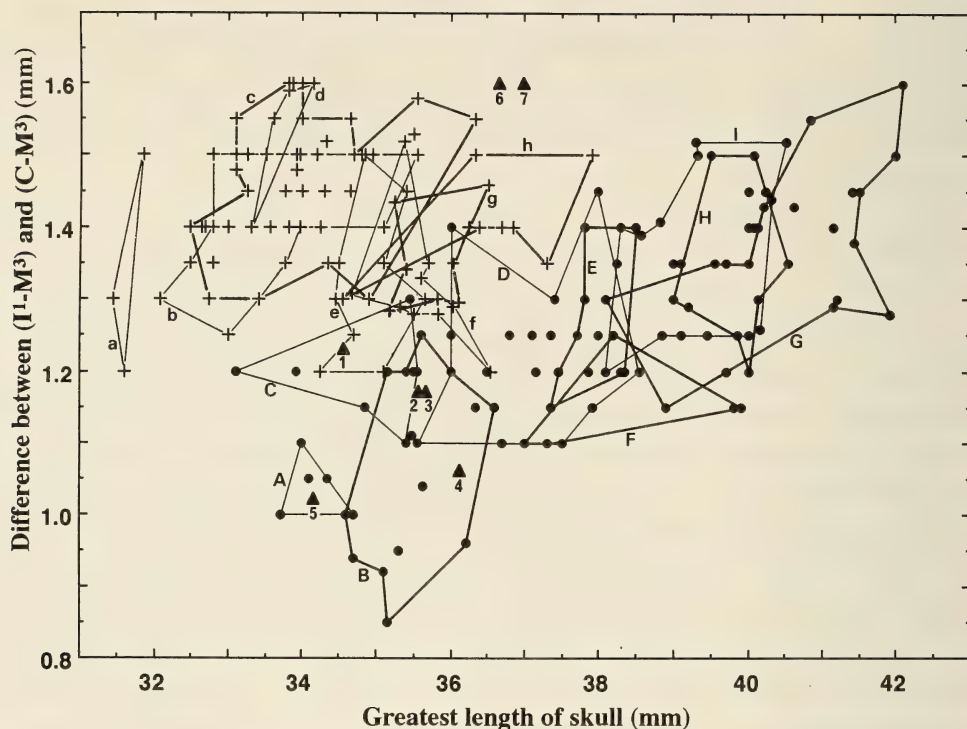
Fig. 1.   The relationship between the difference in length of I^1-M^3 from C-M^3 and the greatest length of the skull of two species of moles (age class I).   For locations V.=village, T.=town, and C.=city.   Closed triangles: paralectotypes of *M. wogura,* 1=RNH16250, 2= RNH16245 (GL was estimated from formula A), 3=RNH28695 (GL from formula A), 4= RNH28696, 5=RNH16249, published as "lectotype" of *Mogera wogura* by Corbet (1978), 6 and 7=RNH28682, GL of the 6 from formula B, GL of the 7 from formula A; closed circles: moles from the southern half of the Japanese main islands (*M. wogura*, Abe 1995), A=Tanegashima, B=Tsushima, C=Yakushima, D=Kyushu, E=Hiroshima Prefecture, F=Tokushima Prefecture, G=Ina Valley including Iida C., Chiyo V., Shiojiri C., Tatsuno T., Nagano Prefecture, and Iwata C., Gotenba C., Shizuoka Prefecture, H=Okinoshima, I=Kiso Valley including Ohkuwa V., Yomikaki V., Agematsu T., Nagano Prefecture, and Inazawa C., Aichi Prefecture; crosses: moles from the northern half of the Japanese main islands (*M. imaizumii* as defined in this paper), a=Mt. Tsurugi, Tokushima Prefecture, b=Iwate and Aomori Prefectures, c=Northeastern Honshu including Fukushima, Niigata, and Ishikawa Prefectures, d= Kiso Valley including Agematsu T., Kisofukushima T., Kiso V., Nagano Prefecture, e=Shiojiri C. including Soga V., Nagano Prefecture, f=Nakano C. including Wada V., Nagano Prefecture, g=Kanto Plain including Tokyo, Tochigi and Ibaraki Prefectures, h=Sendai Plain (Semine), Miyagi Prefecture.

Fig. 2. The relationship between the arcsin-root transformed degree of projection of the incisor row and the greatest length of the skull (age class I). Refer to Fig. 1 for legends.

one. Three paralectotypes (RNH16244, 28694, 28699) in the age class II have arc-like upper incisor row and are included in the group of the southern species in Figs. 3 and 4.

Four aged paralectotypes (ac III and IV, RNH16246-8, 28698) have small differences (0.85-1.11 mm) between I^1-M^3 and C-M^3 and relatively small degrees (25.03-28.66 degree) of projection of the upper incisor row; those of RNH28700 (ac I) and BM43 12 27 5 (ac I) were 1.18 mm, 1.14 mm, 30.85 degree and 28.40 degree, respectively. All these data agree with the diagnostic characters of the southern species.

DISCUSSION

One of us (HA) found that the specimen RNH28684 carried a label noted as the "lectotype". This finding does not agree with the lectotype designation by Corbet (1978). Concerning the lectotype designation of *M. wogura*, Corbet (1978) stated as follows: "..... I therefore select specimen *d*, also numbered 16249, as the lectotype of *Talpa wogura* Temminck. The skull of this specimen has been removed and has the following measurements: upper tooth-row
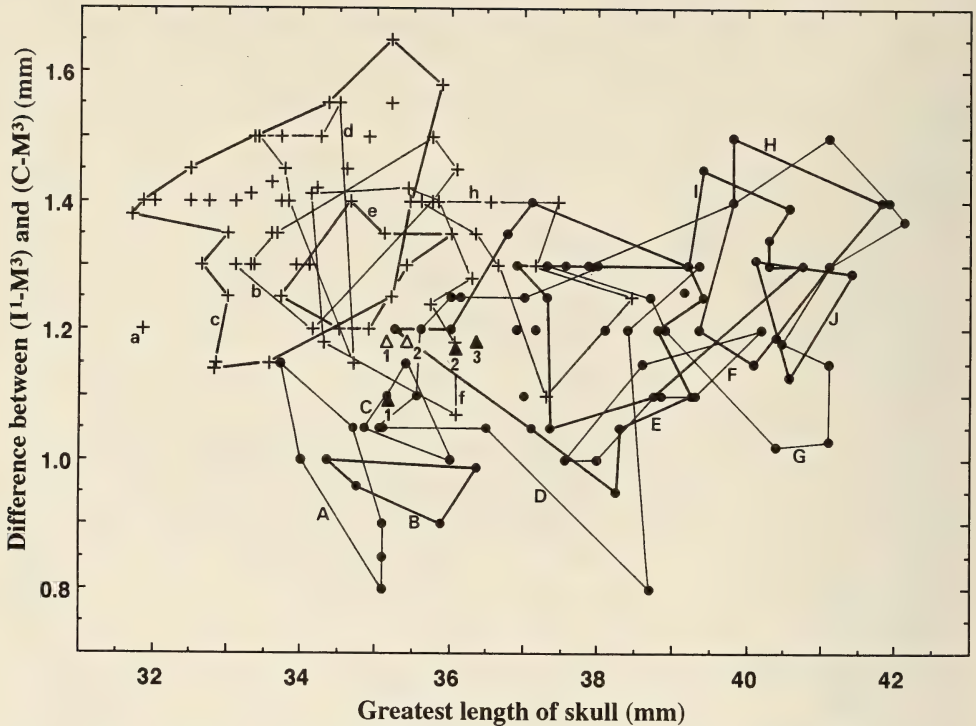
Fig. 3.   The relationship of the difference between I¹-M³ and C-M³ and the greatest length of the skull in the two species of moles (age class II).   Open triangles : RNH28684, intended as lectotype of *M. wogura* by Corbet (1978), GL of the 1 was estimated from formula B, GL of the 2 from formula A ; closed triangles : paralectotypes of *M. wogura*, 1=RNH28694, GL from formula A, 2=RNH28699, GL from formula A, 3=RNH16244 ; closed circles : moles from the southern half of the Japanese main islands (*M. wogura*, Abe 1995), refer to Fig. 1 for A-I, J=Nara Prefecture.   Refer to Fig. 1 for crosses.

14.3 mm ; length of mandible 22.5 mm." This measurements almost complete-ly agree with those (14.34 mm and 22.36 mm, measurement by HA) of RNH28684. On the other hand, those of RNH16249 are 13.87 mm and 21.82 mm (by HA), respectively, which are apparently different from those given by Corbet (1978). Corbet (1978), at this time, did not give a measurement of GL or the condylobasal length which is usually employed as a size character, probably because of the broken skull which he measured. As mentioned earlier, the skull of RNH28684 is partly broken, while that of RNH16249 is perfectly reserved. Thus, it is sure that he intended to designate RNH28684 as the lectotype of *M. wogura.* This intermingled designation of the lectotype by Corbet (1978) might be caused by *"d"*-designated two specimens : RNH16249 (*d* : skull and skeleton, Jentink 1887) and RNH28684 (*d* : skull and skin, Jentink 1888).

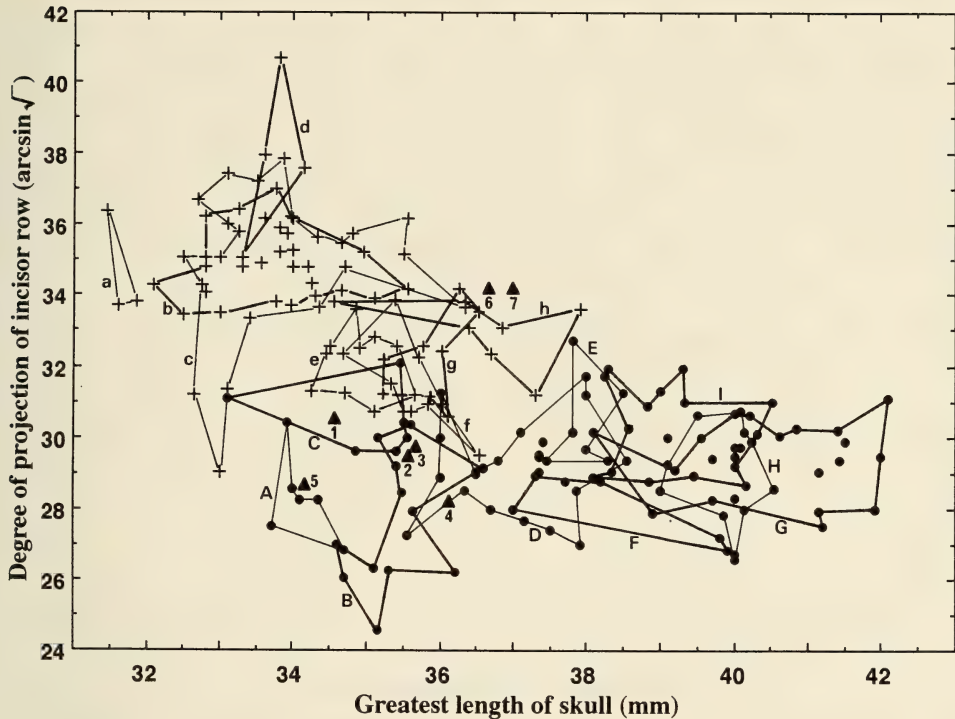In spite of the original intention of Corbet (1978), RNH16249 is regarded as
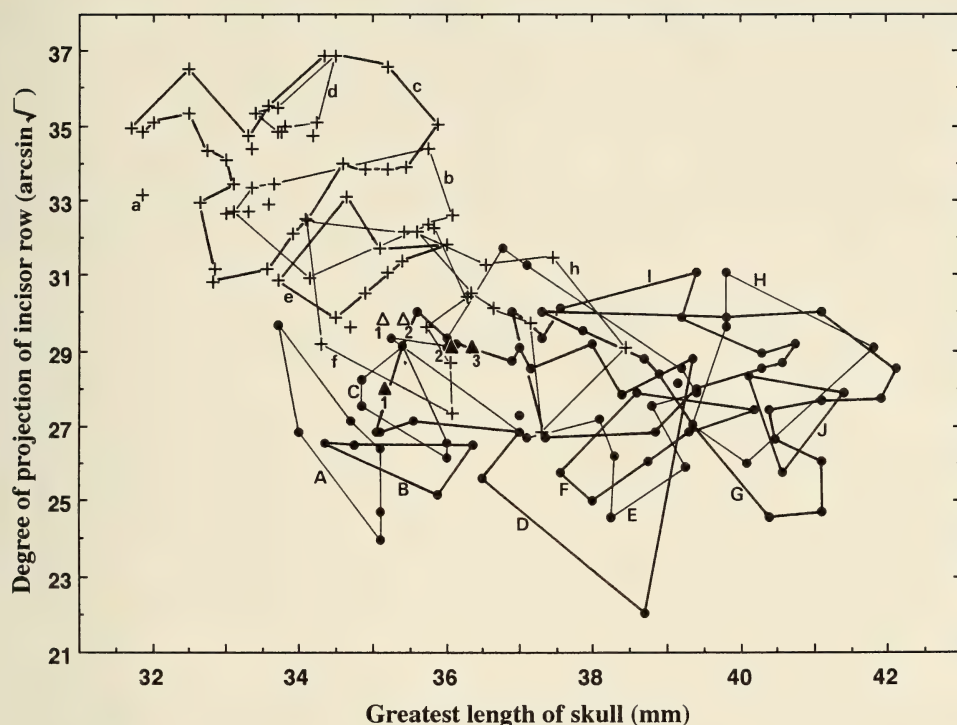
Fig. 4. The relationship between the arcsin-root transformed degree of projection of the incisor row and the greatest length of the skull (age class II). Refer to Fig. 3 for legends.

the lectotype, unless a correction of Corbet's designation is made to exactly indicate RNH28684 as the lectotype. Here we do correct the confusion and confirm RNH28684, for which Corbet (1978) described the set of skin and skull with some measurements, to be the lectotype of *M. wogura*. The two diagnostic characters of the lectotype are somewhat marginal for the southern species but the arc-like shape of the upper incisor row, another diagnostic character, well coincides with that of the southern species. Thus the southern larger moles in Japan are certainly identified as *M. wogura* as concluded in Abe (1995).

The largest skull specimen (RNH28682, ac I) of which the estimated GL is 37.00 mm by formula A or 36.67 mm by formula B, coincides or does not contradict in two characteristics of the upper incisor row with the northern mole species (Figs. 1 and 2). However, the arc-like arrangement of upper incisor row of RNH28682 resembles further that of young specimens of the southern species rather than that of young specimens of the northern species. In the northern species, this type of upper incisor row is found only in older specimens (Abe 1967). The large value of projection degree of upper unicuspid row might be produced by the relatively small measurements of C–M³ due to the broken canines of RNH28682. This skull retains only roots of both canines

lacking crown part of the teeth.   Thus the extraordinary values expressed in Figs. 1 and 2 must be an artifact in the measurement of C-M³.   Consequently, RNH28682 is also identified as one of the southern species.

As discussed by Abe (1995) the northern species had been called *M. wogura* by some taxonomists including Imaizumi (1949, 1960, 1970), Abe (1967) and Hutterer (1993), since Kuroda (1940) erroneously assigned Yokohama, Honshu as the type locality of *M. wogura*.   Abe (1995) corrected this confusion and used *M. minor* as the name of this mole, which had been originally described by Kuroda (1936) as *M. wogura minor* for a small local form from Shiobara, northern Kanto in the range of the northern species.   However, it became clear that the above treatment adopting *M. minor* was not correct referring to ICZN (1985).   After Schwarz (1948) and Ellerman and Morrison-Scott (1951) made lumping of *Mogera* spp. into *Talpa micrura*, Kuroda (1957) renamed *T. micrura minor* as *T. micrura imaizumii* to revise the "*minor*" preoccupied in *Talpa europaea* var. *minor* Freudenberg, 1914.   From this procedure only the name of *M. imaizumii* (Kuroda, 1957) became the valid name for the northern species according to the Article 59b of ICZN (1985).   Thus, *M. wogura minor* Kuroda, 1936 and then *M. minor* Kuroda, 1936 of Abe (1995) had been completely invalid at the time when the third edition (1985) of ICZN was published.   Concerning to *Mogera wogura gracilis* from Nikko, central Honshu (Kishida 1936), no valid description has been known.

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Variation of the mitochondrial DNA and the nuclear ribosomal DNA in the striped field mouse *Apodemus agrarius* on the mainland and offshore islands of South Korea

Sang Hoon HAN[1], Shigeharu WAKANA[2], Hitoshi SUZUKI[3], Yasukazu HIRAI[4] and Kimiyuki TSUCHIYA[1]*

[1]*Experimental Animal Center, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan*
[2]*Department of Genetics, Central Institute for Experimental Animals, Nogawa, Miyamae-ku, Kawasaki 213, Japan*
[3]*Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Kita-ku, Sapporo 060, Japan*
[4]*Institute of Plant Breeding, Tokyo University of Agriculture, Tokyo 156, Japan*
[1]*Present address: Institute of Applied Zoology, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan)*
*To whom correspondence should be addressed
Fax. 0985-85-6951, e-mail. tsuchiya @ post1. miyazaki-med. ac. jp

Abstract.   Restriction fragment variations in nuclear ribosomal DNA (rDNA) spacers, and in mitochondrial DNA (mtDNA), were examined in a total of 14 individuals of the two Korean subspecies of the striped field mouse : *Apodemus agrarius coreae,* collected from the mainland and Jindo and Geoje islands, and *A. a. chejuensis* collected from Cheju Island.   Analysis of heterogeneity in rDNA spacers with ten restriction enzymes, showed that the main Korean populations of *A. a. coreae* have a similar genetic background irrespective of their geographic locality.   In the population from Cheju Island, however, an accumulation of a specific variation, a new SacI site within the internal spacer region of rDNA, was observed.   In the contrast, analysis of heterogeneity of mtDNA with ten restriction enzymes, revealed that mtDNA haplotypes from the offshore islands were distinct from one another and distinct from those of the mainland, with up to 4% of sequence divergence, which corresponds to 1-2 million years of divergence time.   It is suggested that certain geographic conditions, such as the existence of a large number of small islands, may help preserve various mtDNA haplotypes which diverged many millenial ago.

Key words.   *Apodemus agrarius,* mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), ribosomal DNA (rDNA), striped field mouse.

Striped field mice, *Apodemus agrarius*, are widely distributed from north-east Europe to East Asia, including the Korean Peninsula and the island of Taiwan. Two subspecies are represented in South Korea, *A. a. coreae* of the mainland

and numerous offshore islands, and the endemic *A. a. chejuensis* of Cheju Island (Cheju-do) a large island in the Korean Straits (Jones and Johnson 1965). Although genetic characterization is required to elucidate intra-specific variation, only a few reports concerning karyotypic (Tsuchiya 1984), isozymal (Tsuchiya 1984), and mitochondrial DNA (mtDNA) variation (Koh *et al.* 1993) are available at present.   In the last two decades, both intra- and inter-specific genetic analysis have been performed at the DNA level, based on restriction enzyme fragment length polymorphism (RFLP), for nuclear genomic ribosomal DNA (rDNA) (Arnheim *et al.* 1980, Wilson *et al.* 1984, Hillis and Davis 1986, 1988, Suzuki *et al.* 1986, 1987, 1990, Allard and Honeycutt 1991), and for cytoplasmic mtDNA (Yonekawa *et al.* 1981, 1988, Ferris *et al.* 1983).   The rRNA loci exist as a multigene family which consists of several hundred copies in the animal genome.   Each repeating unit of rDNA is composed of three rRNA genes, namely those for 28S, 5.8S, and 18S RNA, which are separated from each other by spacers.   The spacers are known to evolve rapidly and exhibit considerable RFLP between populations and species (Arnheim 1983).   Most of the mutations, recognized by Southern blot analysis, have been fixed to yield-specific repeating unit types (repetypes) within populations or species during the course of their differentiation.   Since each of the restriction sites evolves both in concert and independently (Suzuki *et al*. 1994), data for a set of variations of such sites reflects reproductive divergence of populations and such data are useful for the evaluation of genetic relationships.   In contrast, variation in mtDNA occurs independently of the divergence of populations. Because of the lack of recombination between different mtDNA, and because of the lack of evidence for the existence of wandering males, in some cases a population may include considerably differentiated haplotypes, which had already diverged before the particular populations had diverged.   In other cases, mtDNA may also shed light on unknown historical aspects of populations.   In the case of the Japanese house mice, for example, mtDNA had an ancient haplotype prior to the invasion of the Japanese archipelago (Yonekawa *et al*. 1981, 1988), whereas in the case of house mice in Denmark, only mtDNA from other subspecies spread to the population (Ferris *et al*. 1983).   In this study we compared RFLPs of both rDNA and mtDNA from several populations of two subspecies of *A. agrarius* from South Korea.   From the variations in the nuclear rDNA, we concluded that although the two subspecies are clearly very closely related to one another, they are genetically different.   We also discovered that there are several distinct haplotypes of mtDNA among the populations of *A. a. coreae* indicating that they have a somewhat complex evolutionary history.

MATERIALS AND METHODS

1. Animals

Fourteen Korean striped field mice were collected for use in this study from eight different localities on the South Korean mainland and adjacent islands,

Fig. 1.   Localities from which individuals *Apodemus agrarius* were collected.

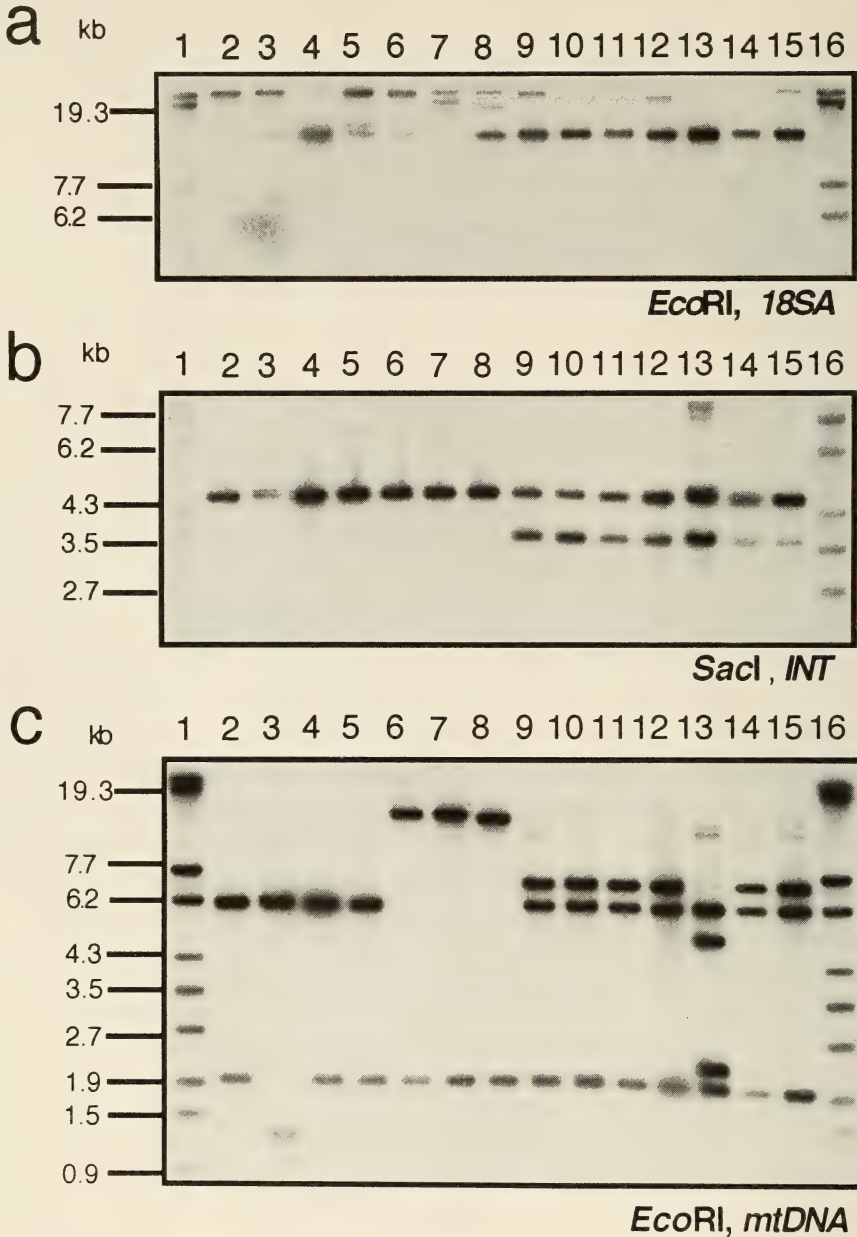Fig. 2.   Southern blot patterns of DNA cleaved with *Eco*RI (a and c), and *Sac*I (b).   The probes were 0.9-kb 18SA (a and b) and whole mtDNA (c).   Refer to Fig. 3 for locations of the probes of rDNA.   Individuals from Gan sung (lanes 2, 3), Yang san (lanes 4, 5), Geoje (lanes 6, 7), Jindo (lane 8), and Cheju (lanes 9–15) islands are compared.   Lanes 1 and 16 show *Eco*T14I digests of λ phage DNA used as molecular markers.

the heights above sea level of these localities were 370 m, 70 m, 30 m, 30 m, 30 m, 980 m, 1280 m and 1700 m, for site numbered 1-8, respectively (see Fig. 1). On Cheju-do samples were collected from four different points.

2. Blot Analysis

Nuclear DNA was prepared from liver samples, as described by Maniatis *et al.* (1982), then southern blot analysis was carried out according to Suzuki *et al.*'s (1990) method. Genomic DNA samples were subjected to digestion with ten restriction enzymes (*Aat*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sac*I, and *Xba*I) for rDNA analysis. For mtDNA analysis, digestion was by means of *Apa*I, *Aat*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, and *Sac*I (*Xba*I was not used). Digested DNA (on nylon filters) was hybridized sequentially with three ³²P-labeled rDNA probes, 18SB, 28S, and INT, and with complete mtDNA. Such sequential hybridization improves the accuracy of the measurement of fragment size, improves the confirmation of complete DNA-digestion and also minimizes laborious work as well as reducing various artificial errors. The rDNA probes (see Fig. 2) were prepared from clones of mouse rDNA, following Kominami *et al.* (1981, 1982). The mtDNA probe was prepared from rat liver, as described by Wakana *et al.* (1986).

3. Construction of Phylogenetic Trees

We began by comparing the restriction cleavage patterns between pairs of mtDNA haplotypes (Table 1) and by counting the different fragments and the fragments in common. Employing a method developed by Gotoh *et al.* (1979), in which both backward and parallel mutations are taken into account (Jukes and Cantor 1969), we were then able to produce a matrix of sequence divergence (Table 2) for all possible combinations of haplotypes (Table 1). We constructed phylogenetic trees for both the unweighted pair-group (UPGMA ; Sokal and Michener 1958) and the neighbor-joining (NJ ; Saitou and Nei 1987) methods. This was possible thanks to a computer program (NEIGHBOR in PHYLIP 3.5c) developed by Felsenstein (1993). From the information relating to the presence or absence of each restriction fragment (Table 1), we were also able to produce a phylogenetic tree for maximum parsimony. For this we used the MIX program, with a "Wagner" option, in the PHYLIP package. Confidence levels for each grouping were calculated by using a bootstrap program (SEQBOOT), with 500 replicates, in the PHYLIP package. The tree itself was produced using the CONSENCE program in the PHYLIP package.

RESULTS

1. Heterogeneity in rDNA spacers

Examples of autoradiographic pictures of blotting with the rDNA probe 18SA can be seen in Fig. 2a and b. From the patterns of the Southern blotting, we constructed restriction maps for the coding and spacer regions of genes for rRNA (Fig. 3). These maps coincided well with the major types of rDNA

Table 1. Presence (1) or absence (0) of the 68 restriction sites of mitochondrial DNA in the ten mtDNA haplotypes in Korean striped field mice, *Apodemus agrarius*.

Haplotype[a]	Population(s)[b] (frequency[c])	*Aat*I	*Apa*I	*Bam*HI	*Bgl*II	*Dra*I	*Eco*RI	*Hind*III	*Pst*I	*Pvu*II	*Sac*I
Aac1	1(1)	00110110	1100	1011	1000110	1100100011	0001100011	101010100	1001	01001	1000011
Aac2	1(1), 2(1)	10000110	1011	1011	0100111	1100100011	0001100101	101010100	1001	01001	1000011
Aac3	2(1)	10000110	1011	1011	0100111	1101000011	0001100101	101010100	1001	01001	1000011
Aac4	3(1)	00110110	1100	1100	1100100	1100100011	1000000100	100110110	1001	00111	0011111
Aac5	3(1)	01000111	1100	1100	1100100	1100100011	1000000100	100110110	1001	00111	0011111
Aac6	4(1)	10000110	1100	1100	0011011	1000001111	0100000100	100110110	0111	00111	0101011
Aah1	5(1), 7(2)	01000111	1100	1011	0010111	1100100011	0010100100	101010100	1001	10000	1000011
Aah2	6(1), 8(1)	10000110	1100	1011	0010111	1100100011	0010100100	101010100	1001	10000	1000011
Aah3	6(1)	10000110	1100	1011	0010111	1100100011	0010100100	110001001	1001	10000	1000011
Aah4	8(1)	10000110	1100	1011	0010111	0110110011	0000111100	110001001	1001	10000	1000011

[a] Aac and Aah represent haplotypes from *A. agrarius coreae* and *A. a. chejuensis*, respectively.
[b] Numbered as in Fig. 1
[c] Total number of samples observed.

Table 2. Sequence divergence among the ten mitochondrial DNA haplotypes of *Apodemus agrarius* from Korea (Upper right), on the basis of the number of common and different fragments (Lower left).

Haplotypes	Sequence divergence (%)[a]									
	Aac1	Aac2	Aac3	Aac4	Aac5	Aac6	Aah1	Aah2	Aah3	Aah4
Aac1	–	1.1	1.3	2.3	2.8	4.3	1.6	1.6	2.4	2.8
Aac2	27/11	–	0.2	2.9	2.9	3.6	1.4	1.2	1.9	2.4
Aac3	26/13	32/2	–	3.3	3.3	3.6	1.7	1.4	2.2	2.6
Aac4	22/21	20/26	19/28	–	0.4	2.4	3.1	3.1	3.8	4.4
Aac5	20/25	20/26	19/28	31/4	–	2.4	2.5	2.8	3.4	4.0
Aac6	16/33	18/30	18/30	22/22	22/22	–	3.4	3.1	3.8	4.4
Aah1	24/15	25/14	24/16	19/26	21/22	18/28	–	0.2	0.8	1.4
Aah2	24/15	26/12	25/14	19/26	20/24	19/26	30/2	–	0.6	1.2
Aah3	21/21	23/18	22/20	17/30	18/28	17/30	27/8	28/6	–	0.6
Aah4	20/25	22/22	21/24	16/34	17/32	16/34	25/14	26/12	29/6	–

[a] Sequence divergences calculated according to Gotoh *et al*. (1979).

repeating units (repetype) of *A. agrarius* previously constructed by Suzuki *et al.* (1990). Among the 26–27 restriction sites examined, these were an *Eco*RI site in the spacer upstream of the 18S rRNA gene (Fig. 2a), an *Aat*I site in the internal spacers, three were polymorphic both within and between individuals, and a *Dra*I site in the spacer downstream of the 28S rRNA gene. These kinds of polymorphism were observed in both subspecies and thus were presumed to have occured before subspecific differentiation. These polymorphic sites were likely to have been subjected to random and independent fixation processes, as observed in the natural populations of the Japanese field mouse, *A. speciosus* (Suzuki *et al.* 1994). In contrast, polymorphism in a *Sac*I site on the internal spacers was consistently and specifically observed in the genomes of individuals of *A. a. chejuensis* (Fig. 2b). Since the apparent differences between the two subspecies are confined to this variation, it may be concluded that the *A. a. coreae* and *A. a. chejuensis* have similar genomic constitutions, but have
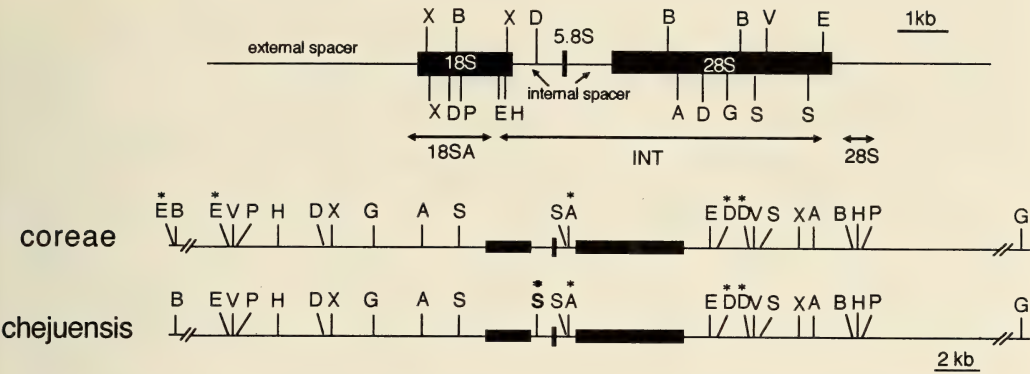
Fig. 3. Restriction maps of the major rDNA repetypes of *Apodemus agrarius coreae* and *A. a. chejuensis*. With respect to the restriction sites on the flanking spacers, only those nearest to the distal end of the genes for 18S and 28S RNA are shown. The top diagram shows the conserved restriction sites in the coding and the internal spacer regions of the gene for 18S and 28S RNA, which are not represented in the lower maps. Probe's positions are shown with arrows. Asterisks indicate polymorphic sites within and between individuals. A = *Aat*I; B = *Bam*HI; D = *Dra*I; E = *Eco*RI; G = *Bgl*II, H = *Hin*dIII; P = *Pst*I; S = *Sac*I; V = *Pvu*II; and X = *Xba*I.

differentiated substantially from each other as far as rDNA-RFLP is concerned.

2. Restriction-fragment patterns of mtDNA

Ten different haplotypes (Aac 1-6 and Aah 1-4) were found in this study (Table 1), their banding patterns, from the Southern blot analysis, with the ten restriction enzymes may be seen in Fig. 3c. There are distinct variations within this species. In particular, individuals from the two offshore islands of Jindo and Goeje, displayed different cleavage patterns from those from all other localities.

To estimate the degree of sequence divergence between haplotypes, we compared site differences between different mtDNA haplotypes. The sequence divergence among mtDNA haplotypes can be estimated from the number of common and of different restriction fragments observed (Table 2). From estimates of the amount of sequence divergence, we constructed two phylogenetic trees for mtDNA haplotypes using both the UPGMA and NJ (Fig. 4a) methods. Additionally, by considering the presence or absence of each of 68 restriction fragments (Table 1), we were also able to construct a phylogenetic tree by the maximum parsimony method (Fig. 4b). The topology of the parsimony tree was identical to that of the UPGMA tree and almost identical to that of the NJ tree. The ten haplotypes were clustered into four groups; Aac 1-3 from the Korean mainland, Aah 1-4 from Cheju-do, Aac 4 and Aac 5 from Geoje Island, and Aac 6 from Jindo Island. In contrast with the rDNA data, the mtDNA haplotypes of *A. a. coreae* were remarkably differentiated, showing the greatest sequence divergence, of 4.3%, between Aac1 and
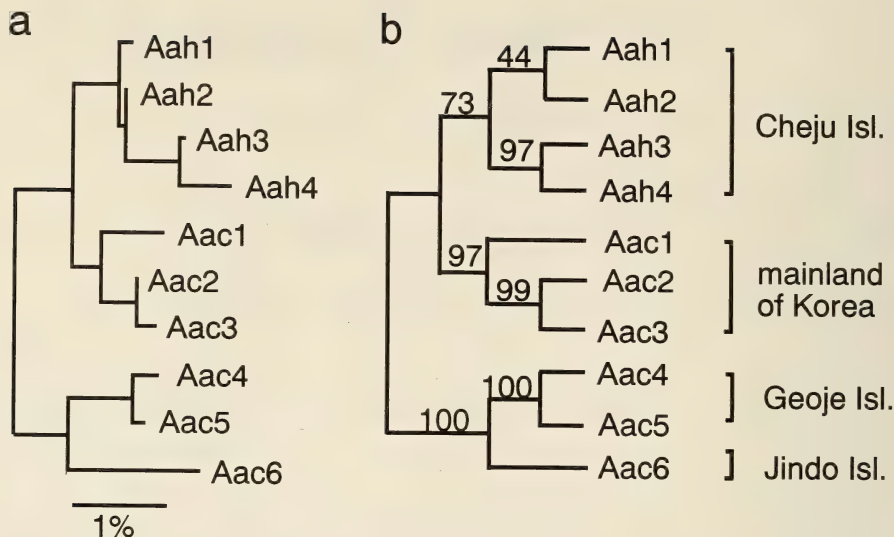
Fig. 4. NJ phylogenetic tree (a) and parsimony tree (b) for the ten haplotypes of mtDNA from *A. agrarius* collected from the Korean mainland, and from Cheju, Geoje, and Jindo islands. The bar below the NJ tree indicates 1% corrected sequence divergence. The bootstrap percentages are given for the maximum parsimony tree. Abbreviations for haplotypes are the same as in Table 1.

Aac6.

DISCUSSION

From a molecular phylogenetic perspective, two conclusions can be drawn from our analyses of RFLP of rDNA and mtDNA. Firstly, the results of RFLP of nuclear rDNA suggest that the degree of genetic divergence within and between the two Korean subspecies of striped field mice, *A. agrarius coreae* and *A. a. chejuensis,* is low. Secondly, the results of the mtDNA RFLP revealed the presence of several distinct mtDNA haplotypes among the various populations, irrespective of their geographic distribution. These observations indicate that Korean striped field mice have similar genetic backgrounds but may have had a somewhat complex history.

From our examination of the rDNA data, we concluded that the extant Korean populations of *A. agrarius* share a similar genetic background. Two subspecies have become slightly differentiated from each other, but only one restriction site (among the 26-27 examined) was observed as a Cheju-specific variation. The new *Sac*I site was observed in approximately half the rDNA repeating units within the genomes of individuals of *A. a. chejuensis.* This level of difference is smaller than that between the two mouse subspecies, *Mus musculus domesticus* and *M. m. musculus,* in which four out of 20 sites examined have differentiated substantially (Suzuki *et al.* unpublished data). Our conclu-

sion, that the genetic backgrounds of the two Korean subspecies of *A. agrarius* are generally similar though slightly differentiated, is consistent with the conclusions of other authors. These two subspecies differ in body size (Jones and Johnson 1965) and in their electrophoretic patterns of transferrin (Tsuchiya 1984), but they are similar in karyotypes (Tsuchiya 1984). Our conclusion is also compatible with geographical evidence indicating that the final isolation of Cheju-do, from the mainland of the Korean Peninsula, occurred only 10,000–20,000 years ago (Park 1988, Ohshima 1990).

In contrast with the rDNA data, cleavage patterns of mtDNA by restriction endonuclease digestion, revealed unexpected patterns. It was found that the Korean populations of *A. agrarius* contain several distinct mtDNA haplotypes, as shown in Tables 1 and 2. Koh *et al.* (1993), working with populations from the Korean mainland, have also observed considerable differentiation in mtDNA haplotypes, ranging from 0.2% to 2.3% sequence divergence. Interestingly, our data revealed that the haplotypes of individual mice from the two offshore islands of Jindo and Geoje, were distinct from those of the mainland, even though these islands are geographically close to the mainland and thought only to have been finally isolated from the Korean Peninsula within the last 10,000 years (Park 1988). The divergence between the two different groups of mtDNA is very large, with sequence divergence of up to 4%, corresponding to divergence times of 1–2 million years, if the evolutionary rate of mtDNA is accepted to be 2–4% per million years (Wilson *et al.* 1985). It is not clear why such highly differentiated mtDNA haplotypes exist, in particular, on the offshore islands, however, there appear to be two possible explanations. Firstly, 1–2 million years ago may have already become differentiated ancestral Korean populations of *A. agrarius* and their distinctive mtDNA has merely been maintained on the offshore islands which were periodically isolated during the last ice age. During each period when the islands were connected to the Korean mainland, mtDNA haplotypes may have been mixed among individuals from the whole area of the Korean Peninsula, and then during subsequent isolation, just one mtDNA haplotype may have become fixed on each of the offshore islands. Korea has many such offshore islands and thus there are many opportunities to maintain many haplotypes of mtDNA. Secondly, it is possible that some of the distinct haplotypes may have migrated from other regions of the world. *A. agrarius* is so widely distributed that individuals from other areas may have been able to contribute to the accumulation of such extensive heterogeneity of mtDNA in Korea. Although we do not have sufficient data on mtDNA haplotypes from other pairs of the world, our preliminary investigations show, however, that these Korean haplotypes are not related to any mtDNA from individuals collected from China, Taiwan, Russia, or Germany (Suzuki *et al.* unpublished data). Thus, it seems most likely that the distinct haplotypes observed in Korea were generated there during the last ice age.

Another interesting issue is the amount of heterogeneity of mtDNA from Cheju-do. The mtDNA haplotypes from Cheju-do were related to one another,

but showed relatively high sequence divergences of up to 1.4% (Aah1 and Aah4 ; Table 2).   The results indicate that mtDNA started diverging at least 0.4-0.7 million years ago.   Because these forms of mtDNA are absent from the other Korean localities examined so far, it is strongly suggested that *A. agrarius* was already distributed on Cheju-do, and probably also on the Korean Peninsula, at least by the middle of the Pleistocene.   It remains uncertain, however, how such divergent mtDNA haplotypes have survived on this small island of just 1819 km².   Distinct haplotypes were even found at the same collection points, and a particular haplotype was found at several different localities.   For examples, haplotype Aah 2 was collected at localty 6 (980 m above sea level) and at locality 8 (1700 m above sea level) on Mt. Halla (see Fig. 1).   Thus, it may be concluded, that there are no significant biogeographic barriers on Cheju-do, and that no significant "bottle-neck event" has occurred in popula-tions of *A. a. chejuensis* during the last half million years.

In general, mtDNA phylogeny does not always reflect the true phylogeny of either populations or species.   As found in this study, mtDNA from Korean *A. agrarius* also showed such intrinsic patterns without consistency, either in the time of divergence or in geographic distribution.   Our data may, however, provide some clues as to the reasons for the high degree of intra-specific mtDNA differentiation.   In the case of Korean *A. agrarius,* the intrinsic geo-graphic distribution of the mtDNA haplotypes may be due to the random dispersion of mtDNA which diverged many millenial ago, furthermore, the existence of numerous offshore islands around South Korea may have helped maintain such differentiated mtDNA.   In order to clarify this issue, further examinations of samples collected from Korea, as well as samples collected from other countries are necessary.

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Foraging behavior of red foxes *Vulpes vulpes schrencki* utilizing human food in the Shiretoko National Park, Hokkaido

Hideharu Tsukada* and Nariaki Nonaka[1]

Regional Sciences, Faculty of Letters, Hokkaido University, N10 W7 Kita-ku, Sapporo 060, Japan,
[1]Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 Kita-ku, Sapporo 060, Japan

Abstract. The utilization of human food (provisions) by red foxes, *Vulpes vulpes schrencki*, in the Shiretoko National Park was investigated to clarify the significance of begging behavior in a natural habitat. An analysis of 736 scats showed that foxes ate prey, such as rodents, insects, fruits, birds and deer, mainly in relation to their seasonal availability. The tendency to depend on a single dietary component increased in the latter half of the tourist season, when many tourists fed foxes, and was lower during the non-tourist season and the first half of the tourist season. The monthly variation in the utilization of provisions did not correlate with availability, and was negatively correlated with the increase in other single dietary components during the tourist season. During the non-tourist season, when relatively little natural food was available, foxes expended great energy to obtain provisions. It is concluded that red foxes in the Shiretoko NP, utilize provisions as a secondary food supply. Such food could be critical for them, however, in order to compensate for the lack of their major natural food resources at certain times of the year.

Red foxes, *Vulpes vulpes,* have a wide ranging diet, enabling them to survive in various environments. They are also flexible in their foraging behavior, changing to cope with the variation in the availability of each food item, as determined by their distribution, and abundance. One example of their flexibility is the development of begging, appearing in front of humans and waiting for them to provide food.

In heavily human-influenced habitats, scavenging enables foxes to access the abundant food source in the form of human waste, begging allows access to additional supplies actually given by people. In England, for example, it is well known that some urban residents actually feed foxes (Macdonald 1987),

*Present address : Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 Kita-ku, Sapporo 060, Japan
Fax. 011-717-7569, e-mail. tsuka @ vetmed. hokudai. ac. jp

and in some cities in Hokkaido, Japan, foxes beg for food (Watanabe 1996). Hence, begging for food is a profitable strategy in areas inhabited by people. Of particular interest, however, is that foxes in more natural habitats also develop this strategy.   In the Shiretoko National Park (Shiretoko NP), one of the most famous natural ecosystems protected in Japan, red foxes have been observed begging for food since 1970 (Tsukada 1994, Watanabe and Tsukada 1996).   Tsukada (1994) indicated that begging was acquired by foxes through interactions with humans during their early lives, however, neither the factors which lead foxes to beg, nor the influence of the development of begging behavior on the utilization of natural food, have been clearly understood.

   In this study, seasonal changes in the frequency of begging, and its relationship to human and natural food availability, were analyzed in order to clarify the importance of begging by foxes living in natural habitat.

MATERIALS AND METHODS

1. Study Area

   The study was conducted in the Shiretoko NP, eastern Hokkaido (Fig. 1), where the mean annual temperature is about 6°C and precipitation is 1100 mm, with winter snow depths reaching 1-2 m in lowland areas.   The park is visited by 15 million tourists every year.   This intensive study was conducted along the main tourist road in the park, the Shiretoko Park Road.   This road has two

Fig. 1   Map of the study area.

gates, which are closed during winter. Gate A is open from May to November, enabling tourists to visit the south-west section of the road, and Gate B is open from June to October, enabling tourists to reach Point "E" (Fig. 1). The vegetation of the area is comprised of mixed broad-leaved and coniferous forests, with an admixture of the wild cherries, *Prunus ssiori* and *Prunus sargentii*, and lianes, such as the Tore vine, *Actinidia arguta*, and the wild grape, *Vitis coignetiae*, which occur at the edge of the forest.

2. Capturing and Identification of Foxes

From 1992 to 1994, forty three red foxes ($\male$18, $\female$25) seen begging for food were captured in the study area, and fitted with individually identifiable colored ear tags (Allflex 25, Allflex New Zealand Ltd.). Foxes were classified into two age groups: juveniles (<1-year-old; $\male$7, $\female$11) and adults ($\geq$1-year-old; $\male$11, $\female$14), by the degree of tooth wear (Harris 1978). Females which were rearing pups during June and July 1993 and 1994 were identified by the development of their nipples.

3. Begging for Food

Begging was defined as "appearing on or along a road in order to obtain food which might be given by humans". Practically, the following criteria were used to identify incidents of begging:
1) a fox appeared on or along the road during the day time when many tourists were likely to be about; and
2) a fox stayed in a position where people in their vehicles could notice them.

Observations were carried out from a car while driving the approximately 20-km-long Shiretoko Park Road (from "S" to "E" in Fig. 1) during the periods from June to October in 1993 and 1994, when both gates were open. This period is henceforth referred to as "the tourist season" and the remainder of the year as "the non-tourist season". The 20 km journey was made once every two hours from 07:00 to 17:00, on two weekdays each month (a total of twenty four trips). For each fox, its frequency of begging each month was calculated by the equation: the number of trips in the month, when begging was observed, divided by the total number of trips in the month with the exception of some juvenile foxes in 1994 which were not individually identified. In addition, the average number of juvenile foxes observed begging per kilometer of the total length of the trips, was calculated for each month in 1993 and 1994.

4. Fecal Analysis

Fox scats, deposited along the Shiretoko Park Road, were collected every month from April 1994 to February 1995, except for December 1994 (sample sizes: April 169, May 100, June 129, July 114, August 39, September 9, October 60, November 89, January and February 36; total 736). After taking samples for parasitological inspection, scats were preserved in a mixture of 1% formalin and 0.3% Tween 20, then sterilized by heating at 70°C for more than eight hours. Samples were then washed through a 0.1 mm mesh sieve. Undigested

items, identified by naked eye, or under a microscope, were weighed, after drying, to the nearest 1 mg. These items were first identified as "animal", "plant" or "other", then divided into broad categories, such as mammal, bird, reptile, fruit, or roughage (non-fruit vegetable matter), and then further classified into 17 narrow categories including all major food items of foxes in eastern Hokkaido (Abe 1975, Yoneda 1982). All items obtained from people were classified as "human food", or "provisions". The percentage occurrence and percentage weight of each food category were calculated (an adjustment for weight lost for parasitological inspection was made). The percentage occurrence of a category shows the relative frequency of that category in all fecal samples. The percentage weight of the same category shows its weight relative to the total weight of all categories.

Previous studies have usually multiplied the dry weight of food items by a coefficient of digestibility in order to estimate the amounts of food actually consumed (Goszczyňki 1974, 1986, Yoneda 1982, Jedrzejewski and Jedrzejewški 1992). In this study, however, the coefficient of digestibility of provisions and other food categories could not be obtained, thus such estimations were not feasible. Therefore, the dietary components of foxes were mainly traced by percentage occurrence. As this method is prone to the bias of under-estimating small food items (Kruuk 1989), we compared percentage occurrence to the results of percentage weight.

5. Estimation of Food Abundance

The availability of the major food sources of foxes in eastern Hokkaido, such as rodents, birds, insects and fruits (Abe 1975, Yoneda 1982), were estimated by the following methods, every month from April to November in 1994.

Rodent abundance was estimated from the number of individuals captured using 25 live traps baited with oats set for three days each month and checked every morning. Traps were set 10 m apart at four sites along the road. The number of rodents captured, excluding recaptures (released after clipping their toes) was recorded, and the number per 100 trap-nights was calculated to provide an index of rodent abundance.

Insect abundance was estimated from the number of terrestrial species collected in 20 baited pitfall traps (7 cm diameter, 13 cm height). Traps, one meter apart along trap lines set perpendicular to the road at four sites in the forest, were set for two days each month. The mean number of insects captured at all sites in each month was calculated and used as an index of abundance.

The relative abundance of fruit was estimated from the numbers of fallen ripe fruits. Forty seven *A. arguta* and *V. coignetiae* vines were selected along the road through the study area, and the numbers of ripe fruit on each vine were monitored. A decline in the number of fruit, after the maximum number was reached, was considered to reflect the availability of fallen fruit. The proportion of fallen fruit, in a given month, was calculated for each vine by the equation : (decrease in fruit numbers in a given month)/(maximum number of

fruit). The average proportion of fallen fruit from the 47 vines was used as an index of relative fruit abundance.

Bird abundance was estimated, based on the work of Nakagawa (1985), and Matsuda (unpubl.). From Nakagawa's (1985) description of the Shiretoko avifauna, and its seasonal change, the monthly species composition of birds in the study area was estimated. Seasonal variation in numbers of each species, was calculated from Matsuda (unpubl.), who censused the numbers of different species of birds in the same study area during the 1992 and 1993 summers (June and July), and the 1993 and 1994 winters (January and February). Matsuda's summer and winter numbers were used as monthly numbers for each species from April to November, and from December to March, respectively. The total number of all species of birds in each month was calculated by summation of the estimated number of each species of bird occurring in the month. This was used as an index of avian abundance.

The availability of provisions was estimated from the number of vehicles passing along the Shiretoko Park Road, because preliminary observation showed that most foxes were fed by tourists traveling by car or coach. Abundance was expressed as the number of vehicles met per minute by investigators on the whole park road in June-October in 1993 and 1994, and on the south-west of the road, from Gate B, during May and November of each year.

For the purposes of this paper, March to May are defined as spring, June to August as summer, September to November as autumn, and December to February as winter.

RESULTS

1. Seasonal change in the frequency of begging during the tourist season

Thirty foxes (20 adults and eight juveniles in 1993; 15 adults and one juvenile in 1994, with some observed in both years) were observed begging for food a total of 557 times. There was no significant difference between the sexes, or between females in differing reproductive conditions, in the mean frequency of begging (Table 1).

Table 1. Frequency of food begging by adult foxes are compared between sexes or between reproductive conditions of female foxes. Mean with SE are given. Sample sizes are shown in parentheses.

	1993	U-test	1994	U-test
Adult males	0.16±0.04 (n=8)	ns	0.13±0.02 (n=4)	ns
Adult females	0.23±0.04 (n=12)	ns	0.15±0.02 (n=11)	ns
Female in reproductive condition	0.23±0.04 (n=9)	ns	0.14±0.03 (n=6)	ns
Female in non-reproductive condition	0.22±0.07 (n=3)		0.19±0.02 (n=4)	

ns: statistically non-siginificant ($p>0.05$)
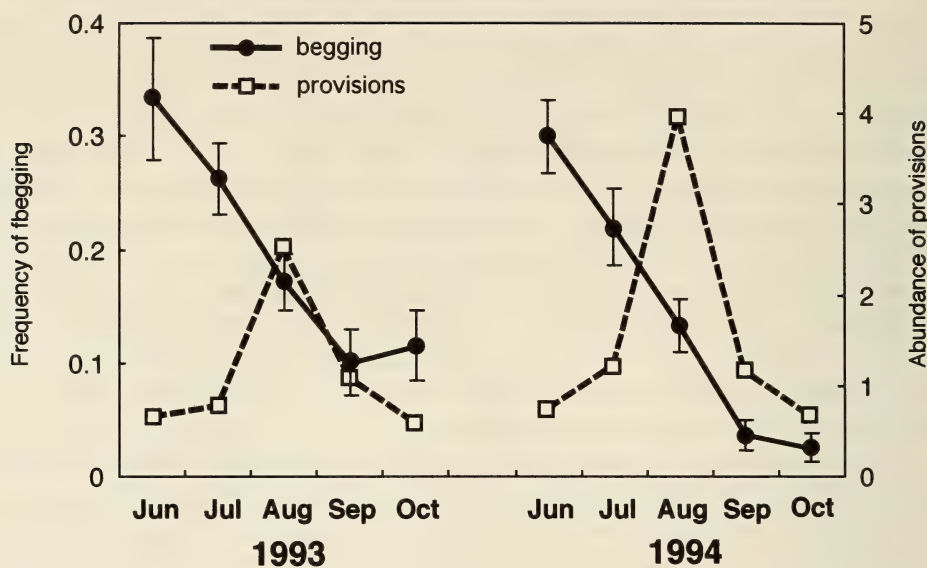
Fig. 2 Monthly variation in the frequency of begging by adult foxes (solid line with circles), and in the abundance of provisions (broken line with squores) in the study area.
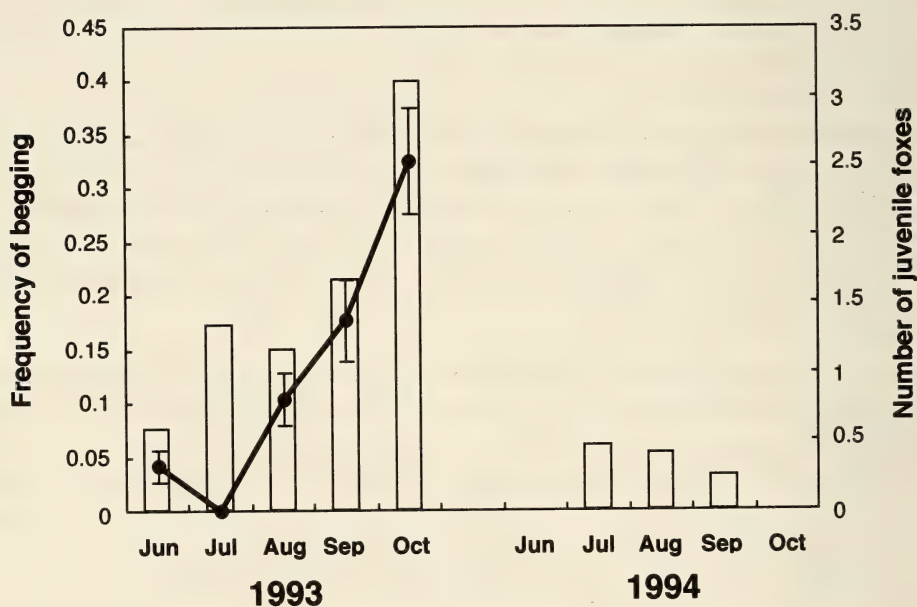

Fig. 3 Monthly variation in the frequency of begging by eight juvenile foxes in 1993 (solid line and circles) and in the average number of juvenile foxes begging per km in 1993 and 1994 (histogram).
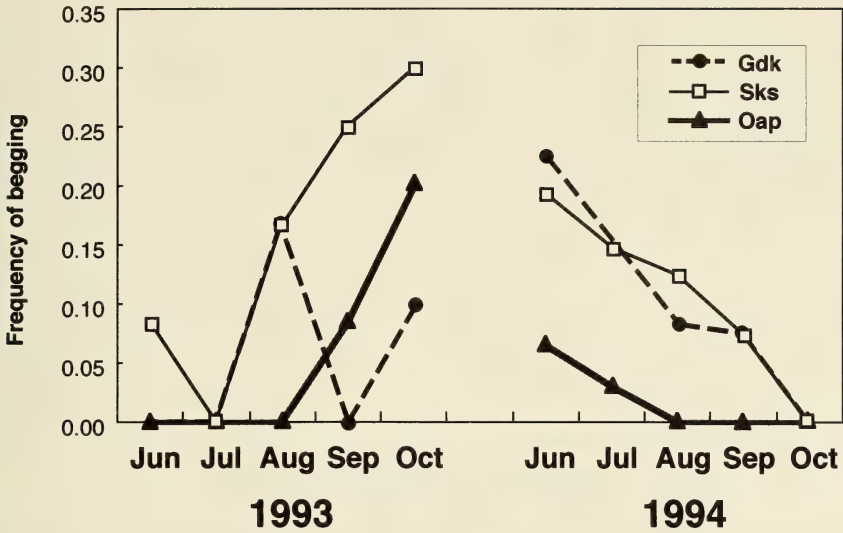
Fig. 4   Monthly variation in the frequency of begging by three foxes born in 1993.

The frequency of begging by adult foxes varied both between and within years.   Among the 12 adults which were observed begging in both 1993 and 1994, the mean frequency of begging was significantly higher in 1993 (0.27 ± 0.03 *SE*) than in 1994 (0.15 ± 0.02 *SE* : Wilcoxon sign rank test, $p < 0.01$).   In both years, however, adults were found to beg most in June and least in autumn (Fig. 2).   This pattern of seasonal change was consistent in both years ($r^s = 0.96$, $p < 0.06$, n=5).

The availability of provisions also varied between and within years.   The mean abundance of provisions was significantly lower in 1993 (1.12 ± 0.32 *SE*), than in 1994 (1.55 ± 0.55 *SE* : Wilcoxon sign rank test, $p < 0.05$).   It increased in summer, peaked in August, and decreased in autumn in both years.   Surprisingly, the frequency of begging did not correlate with the availability of provisions.

As with adults, the mean frequency of begging by juvenile foxes also varied within the year, however, monthly fluctuations were not in phase with adults ; it was, for example, lower in summer and higher in autumn, while the reverse occurred in adults in 1993 (Figs. 2 and 3). In 1994, however, juveniles showed little begging behavior in autumn, and the number of juveniles begging per km was significantly lower than in 1993 (Wilcoxon sign rank test, $p < 0.05$; Fig. 3). Three young foxes born in 1993, also remained in their natal range throughout the 1994 study period.   Their seasonal frequency of begging differed noticeably from that in 1993, and was highly correlated with that of other adult foxes ($r = 0.91$, $p < 0.05$; Fig. 4, cf. Fig. 2).

2. Food habits and food availability

Fecal analysis revealed that in terms of perctage occurrence, six food items ranked highest : roughage, rodents, insects, fruits, birds, and deer. Although roughage (consisting of dry twigs, dry leaves and grasses) occurred most frequently in feces, it was assumed to have been accidentally included in samples when collecting them, or that it had been swallowed with other food by the foxes, because it had not been listed as a staple food in previous fox studies (Abe 1975, Misawa 1979, Yoneda 1982, Kondo *et al*. 1986). Therefore, roughage was excluded in the following analysis. Five of the highest ranking dietary components by weight were : fruits, rodents, insects, deer and birds, which together accounted for 71.0% of the total weight of feces. Hence, the five major foods of Shiretoko foxes, both by percentage occurrence and by percentage weight, were rodents, insects, fruits, birds and deer. Provisions appeared in 11.8% of all fecal samples and accounted for 4.3% of total fecal weight (Table 2).

Table 2. Annual diet composition of fox feces in the Shiretoko National Park (n=736).

Food categories	Occurrence (%)	Weight (%)
Rodents	40.1	12.2
Insects	40.1	11.7
Fruits	26.5	29.5
Birds	22.0	7.7
Deer	16.7	9.9
Fishes	9.5	6.2
Other mammals	5.7	2.5
Unidentified	4.2	1.6
Soil	4.1	5.1
Earthworms	3.4	4.3
Other animals	2.3	0.8
Reptiles	2.2	0.6
Shellfishes	0.5	0.2
Crustcea	0.3	0.1
Fungi	0.3	<0.1
Roughage	44.6	3.3
Human foods	11.8	4.3

The composition of the diet was found to vary with the seasons. The greatest range of food categories found in feces occurred in April, May and June. The range then decreased until September, increased again in October and November, and decreased once more in January and February (Fig. 5). From May to November, just one food category occurred in more than 50% of scats each month. The percentage occurrence of the most frequently occurring category, each month, increased from May to November (with the exception of September, when the sample size was very small ; Fig. 5). Thus, the tendency to depend on a single dietary component increased from spring to
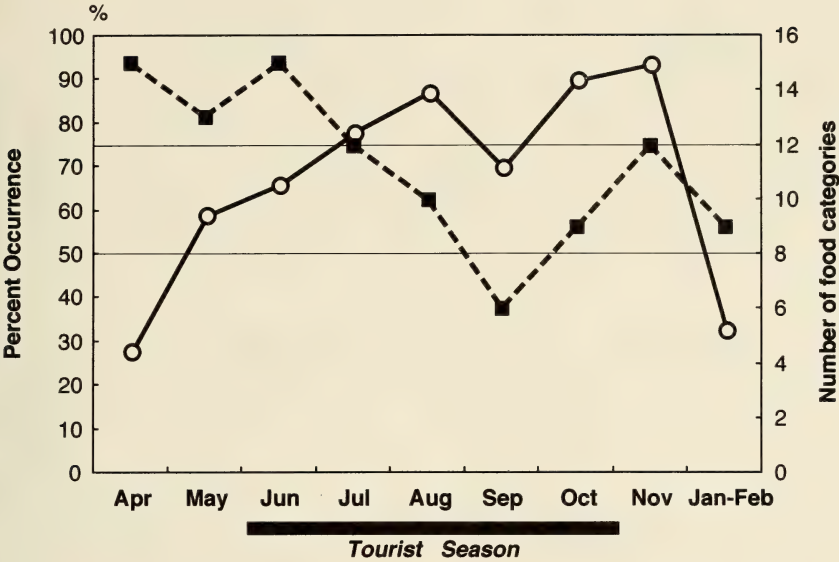
Fig. 5   Seasonal variation in the number of food categories occurring in feces (broken line with squares) and the percent occurrence of a food category showing the highest value each month (solid line with circles).

autumn, and decreased in winter.

The utilization of main food categories also changed seasonally. Fruits, such as *A. arguta, V. coignetiae* and *Prunus* spp., occurred most in autumn, with *A. arguta* in particular, accounting for 87.9% of the total weight of fruits taken. The seasonal variation in both percentage occurrence and percentage weight of fruit in fox feces was correlated with the change in their relative abundance (occurrence : Kendall's $\tau = 0.68$, $p < 0.05$; weight : Kendall's $\tau = 0.58$, $p < 0.05$; Fig. 6A).

Rodents included the northern red-backed vole, *Clethrionomys rufocanus,* and the grey red-backed vole, *C. rutilus,* and two endemic species of field mice *Apodemus speciosus* and *A. argenteus.* Voles occurred in 91.8% of scat samples containing rodents, and accounted for 90.2% of their total weight. Rodents occurred mostly in May, although the highest percentage by weight was in April (Fig. 6B). The abundance of rodents increased sharply from June to August, reaching a peak in October, yet there was no correlation with percentage occurrence in feces (Kendall's $\tau = -0.36$, $p > 0.05$), although there was a negative correlation with percentage weight (Kendall's $\tau = -0.71$, $p < 0.05$; Fig. 6B).

Insects available to foxes included Hymenoptera, Coleoptera, Orthoptera, and various larvae. Coleoptera in particular occurred in 91.8% of scats containing insects, and accounted for 96.4% of their total weight. Most insects occurred in samples collected during summer (Fig. 6C), with their percentage occurrence (Kendall's $\tau = 0.81$, $p < 0.05$; Fig. 6C) in scats correlated with their
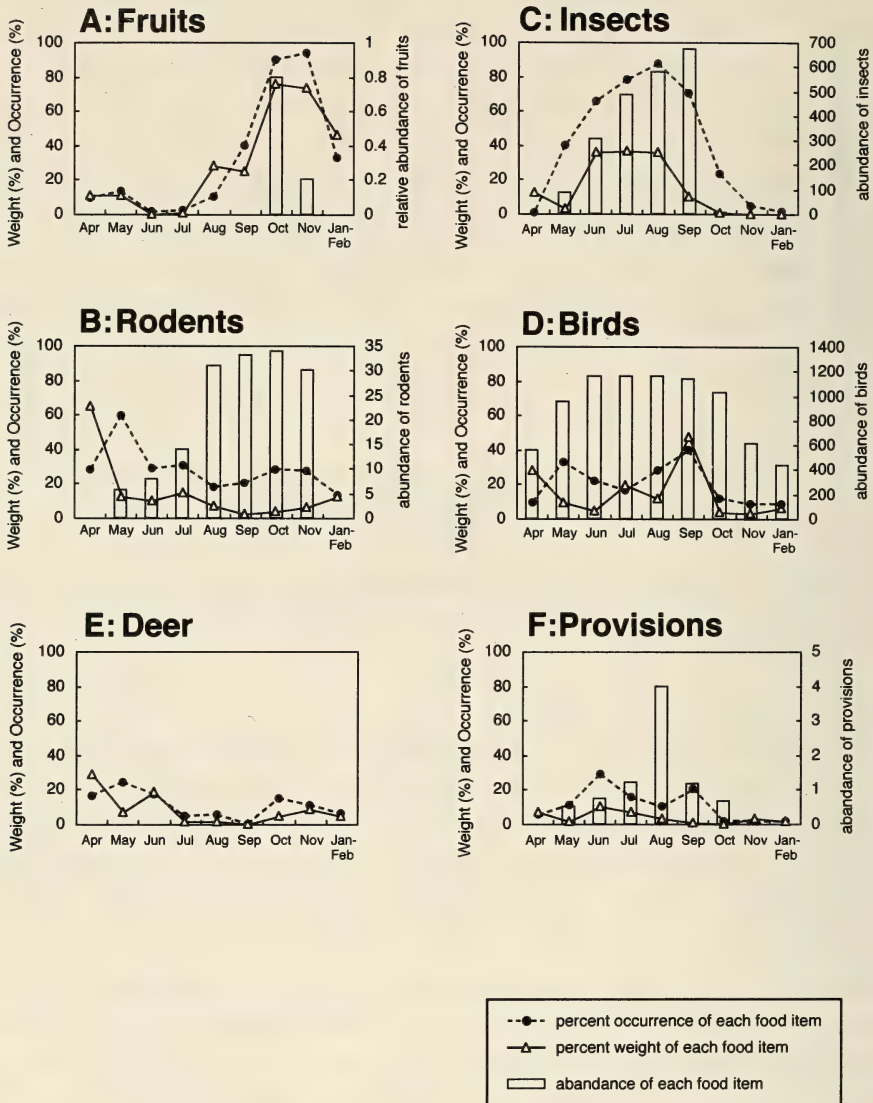
Fig. 6   Seasonal variation in the weight and occurrence of six dietary components and their abundance in the study area.

availability.   The percentage weight, however, did not correlated with their availability (Kendall's $\tau = 0.52$, $p > 0.05$).

Birds were most abundant in May and September, however, neither their percentage occurrence nor their percentage weight in scats correlated with their abundance (occurrence: Kendall's $\tau = 0.42$, $p > 0.05$; weight: Kendall's $\tau = -0.99$, $p > 0.05$) (Fig. 6D).   A few pieces of egg shell were present in samples from May to July.

Sika Deer, *Cervus nippon*, occurred more frequently during April, May and June than in other months (Fig. 6E).   In June, a few calf hooves were present in samples.

Provisions occurred most frequently during the tourist season, in spring and summer, peaking in June (Fig. 6F), and less frequently during the non-tourist season.   During the tourist season, provisions included plastic materials, paper, aluminum foil, and corn.   The frequency of begging by adult foxes during this season, correlated with the percentage weight (Kendall's $\tau=1.0$, $p<0.05$, n$=5$), but did not correlated with the percentage occurence of provisions (Kendall's $\tau=0.6$, $p>0.05$, n$=5$).   This is probably due to the small sample size in September when begging was unexpectedly scarce.   During the tourist season, provisions identified in fox scats were mostly composed of food given by people to begging foxes.   The availability of provisions during the tourist season peaked in August, but did not correlate with either the percentage occurrence or the percentage weight of provisions in scat samples (occurrence : Kendall's $\tau=-0.14$, $p>0.05$; weight : Kendall's $\tau=-0.24$, $p>0.05$; Fig. 6F). The percentage occurrence of provisions in scats each month was found to be negatively correlated with the percentage occurrence of the most frequently occurring food during the tourist season (r$=0.96$, $p<0.01$), and showed a similar tendency in relation to the number of food categories, although the correlation was not significant in this instance, perhaps because of a potential bias in September due to the small sample size (Fig. 5, Fig. 6F).

During the non-tourist season, provisions occurred most in April and May, and household scraps were observed in 64% of feces counting all provisions.

DISCUSSION

Begging by red foxes did not differ between the sexes, or between adults in differing reproductive conditions, thus indicating a general similarity in feeding strategies.   This is in agreement with data on the food habits of hunted foxes from other countries, which also indicated that males and females had similar diets (Englund 1965, Sequiera 1980).

The difference in the frequency of begging, between juvenile and adult foxes changed seasonally.   The frequency of adults begging decreased in autumn in 1993 and 1994, but the frequency of juveniles begging increased only in autumn 1993.   Juvenile foxes were probably fed by their parents until they were 13 weeks old, or until July or August, and they gradually began to feed themselves (Nakazono 1994).   In general, juveniles have inferior hunting skills during their first autumn, therefore, they tend to depend on more easily accessible food than adult foxes (Englund 1969, Sargeant *et al*. 1984).   This would explain the increase in the frequency of begging among juveniles from spring to autumn in 1993, and furthermore, by the following year, 1994 (by when they had become more skillful hunters), three of those same juveniles from 1993 showed the same seasonal change in begging frequency as other older adults.

What was unexpected, however, was a reduction in the frequency of

begging by juvenile foxes from summer to autumn 1994. In October and November 1994 the fruit biomass of *A. arguta* was higher than in an average year (Matsuda pers. comm.), not surprisingly the readily available fruits dominated the diet of the foxes, occurring in 86.6% of scats (n=149). This was significantly higher than 1993 (33.8%, n=157 ; χ^2=88.5, Fisher's exact $p<$ 0.001 ; Tsukada unpubl.). Thus, unlike in autumn 1993, in autumn 1994 juvenile foxes were easily able to depend on these fruits, their abundance probably explaining the decrease in begging in autumn 1994.

The seasonal change in the frequency of begging by adult foxes was similar in both 1993 and 1994. If this change was dependent on food abundance, it should have been positively correlated with changes in the abundance of provisions in each year. Such a correlation, however, was not observed. Furthermore, adults begged less frequently in 1994 than in 1993, whereas conversely provisions were more abundant in 1994 than in 1993, suggesting that there was no relationship between frequency of begging by adults and the availability of provisions. Why didn't begging frequency correlate with either seasonal or annual variation in the abundance of provisions?

According to Calisti *et al.* (1990), and Doncaster *et al.* (1990), the diet of red foxes varies in relation to food availability. The foxes in the Shiretoko NP study area tended to prefer one food category in each season. Such seasonal switching of preferred foods and main food categories is likely to be dependent on their availability. Food availability, however, can be broken down into two important aspects : abundance and ease of acquisition.

Food items such as fruits and terrestrial insects are easily obtainable, thus their availability is directly correlated with abundance. In fact, foxes in the study area chose these foods in relation to their abundance. On the other hand, the availability of active prey, such as live rodents and birds, is dependent on both their abundance and on their ease of acquisition. Rodents and birds were consumed by foxes but not in direct relation to their abundance.

During springs when ground cover, such as snow and grasses, were scarce, and hence rodent vulnerability was high, rodents were eaten frequently (Yoneda 1983, Jedrzejewski and Jedrzejewski 1992). Birds were eaten most during the migration seasons (April and September ; Matsuda pers. comm.), and during the nesting season (May to July), indicating that they were most intensively predated when most vulnerable. The utilization of deer by foxes increased from April to May (the period of highest mortality ; Kaji pers. comm.) ; it was also common in June, the peak birth period for deer on Shiretoko (Yabe 1995). Thus, rodents, birds and deer, major items in the diet of foxes on Shiretoko, were utilized depending on their vulnerability.

Adult foxes were easily able to obtain provisions during the tourist season. Even juvenile foxes, with inferior foraging skills and still mostly dependent on their parents for food, were able to obtain food from people. Therefore, the availability of provisions is considered to be directly correlated with its abundance. The utilization of provisions by foxes in the tourist season, however, did not depend on their availability. In fact, fecal and behavioral analyses

indicated that utilization of provisions was strongly dependent on the utilization of other food items, probably based on their availability. Indeed, the utilization of provisions was negatively correlated with the frequency of the primary dietary component during the tourist season.

A low contribution of a principle dietary component indicates the low availability of any particularly palatable prey. Such deficiencies tended to occur during April, May and June, and also during January and February. During these periods, foxes broaden their diet to include less preferred prey, such as shrews, insectivorous small mammals (Macdonald 1977) which occur in feces only during April (weight: 1.8%; occurrence: 5.3%) and May (weight: 0.3%; occurrence: 1.0%). Of particular interest is that provisions were found more frequently in feces during April, when the park road was closed, than in October, indicating that foxes made lengthy excursions to human residential areas up to 13 km from the locations where feces were collected. Admittedly, such excursions were made by some foxes which begged for food even during the non-tourist season (Tsukada 1994). These particular individuals expended a great deal of energy to obtain provisions when major natural foods were scarce.

Given that foxes in the Shiretoko NP showed no notable inclination towards provisions, even during the tourist season, when the availability of provisions was highest, it appears that provisions were utilized mainly as a secondary food source, when more palatable and preferred natural foods were absent or less abundant. This observation is not unique, as Englund (1965), and Lucherini and Crema (1994) also observed that some human waste were used as a secondary food source in other natural habitats.

The major, previously reported, fox prey items are small rodents, hares and rabbits, wild fruits and berries, insects, and birds (Ables 1975, Lloyd 1980, Sequiera 1980), all of which fluctuate in their abundance, and thus in their availability to foxes. It must be vitally important for foxes to meet the temporal shortages in their major prey. Provisions are generally available year round wherever human activity occurs. Furthermore, in Shiretoko NP, many outdoor recreationists visit natural areas inhabited by foxes and make provisions available to foxes. Provisions seem, therefore, less preferable than natural foods, but provide an alternative when natural foods are in short supply. It is likely that foxes inhabiting a natural area such as Shiretoko NP may begin to beg for provisions simply because they are offered them by the numerous visitors. Provisions may also be a critical food in terms of increasing the carrying capacity of the area normally regulated by natural food availability.

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Short Communication

Conception dates of Sika deer on the Boso Peninsula, central Japan

Masahiko ASADA and Keiji OCHIAI[1]

Laboratory of Forest Zoology, Faculty of Agriculture, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan
[1]*Natural History Museum and Institute, Chiba, Aoba-cho, Chuou-ku, Chiba 260, Japan*
Fax. 03-5800-6895, e-mail. QZE16660 @ niftyserve. or. jp

The seasonal characteristics of mammalian reproduction are partly related to the seasonal dietary conditions of the species concerned (Lincoln 1985, Sadleir 1987, Bronson 1989). Birth and lactation of herbivores typically occur in spring in conjunction with the peak in available vegetation (Bronson 1989). Since this seasonal pattern of food availability varies with latitude, breeding seasons also vary with latitude in, for example, mountain sheep (Bunnell 1982), reindeer (Leader-Williams 1988) and deer of the genus *Odocoileus* (Bronson 1989).

The range of the Sika deer (*Cervus nippon* Temminck) extends along the Asian coastline of the Pacific Ocean from virtually the sub-tropical (14°N) to the sub-arctic regions (50°N) (Ohtaishi 1986, Whitehead 1993). As a consequence, the breeding season of this species is expected to differ at the different latitudes of the great length of its range. So far, however, details of the breeding season of Sika deer have only been reported from Hokkaido (43.5°N, Suzuki *et al*. 1996), Hyogo Prefecture (35°N, Koizumi 1991), and Nara Park (34.4°N, Miura 1984), and more wide-ranging researches are required to elucidate the situation more fully. Here we report an examination of the conception dates of Sika deer on the Boso Peninsula in central Japan (35°N).

STUDY AREAS

The study area, of 124 km², ranges in elevation from sea-level to 300 m above sea level, consists of steep slopes, and is located in Chiba Prefecture, central Japan (35°N, 140°E, Fig. 1). The annual precipitation in the area is 2,000-2,400 mm, and the mean monthly temperature is about 4°C in mid-winter and 25°C in mid-summer (University of Tokyo 1988). The predominant vegetation of the area consists of evergreen broad-leaved forest, primarily *Machilus thunbergii* and *Castanopsis sieboldii,* natural coniferous forest consisting of *Abies firma* and *Tsuga sieboldii,* and plantations of two species of conifers, *Cryptomeria japonica* and *Chamaecyparis obtusa.*

In order to detect intra-population differences, the study area was divided
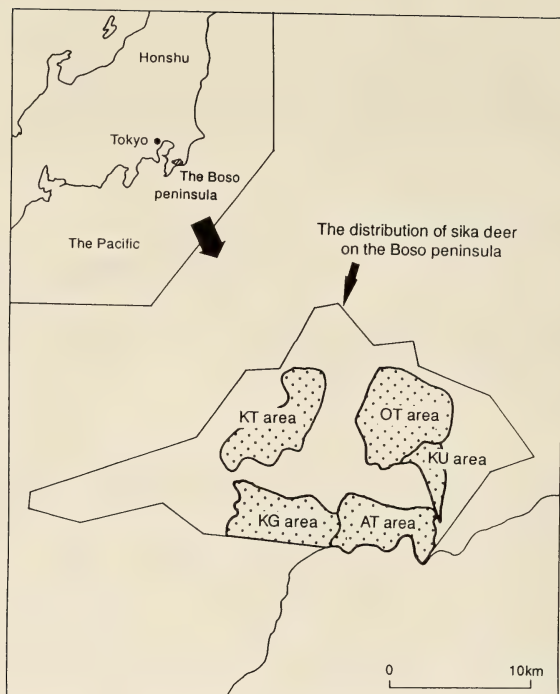
Fig. 1   Study area.

into five sub-areas according to deer density (Fig. 1) : the high density AT area where there were 22.4–37.9 deer/km², and the lower density KG, KU, OT and KT areas where there were : 14.7, 0.9, 1.1–5.7, and 6.7–8.4 deer/km², respectively (Chiba Prefecture and Deer Research Group on Boso 1993).

MATERIALS AND METHODS

Female Sika deer on the Boso Peninsula are regularly culled, as a means of pest control.   From such specimens we collected 180 fetuses in January and February 1993, February and March 1994, and February and March 1995.   The ages of pregnant deer were determined by tooth replacement and by counting the cementum layers of the first incisors (Ohtaishi 1980).

The crown-rump length (CRL) of each fetus was measured to the nearest millimeter and the gestational age was estimated from the linear regression formula proposed by Koizumi (1991) :

$$Y = 50.23 + 0.42X$$

where X equals CRL (mm) and Y equals gestational age (days).   This equation is based on a mean gestation period of 234 days and a body length at parturition of 440 mm as found for the deer population of the Tanzawa Mountains, central Japan (Iimura 1980).   On the Boso Peninsula, the mean gestation period was found by Nakajima (1929) to be 235 days.   The mean shoulder height ± *SD* of

adult females in the Tanzawa Mountains was 77.8 ± 6.7 cm (Iimura 1980) whereas on the Boso Peninsula it was 74.0 ± 3.8 cm (Ochiai and Asada 1995). Since the differences between these two populations were not large, we adopted Koizumi's (1991) model for the Boso Peninsula. The date of conception was estimated from the collection date and the gestational age.

RESULTS AND DISCUSSION

Sika deer conceived between 8 September and 11 December, with a median date of 23-24 September, in all sub-areas of our Boso Peninsula study area. The crown-rump lengths of fetuses collected from the area ranged from 28 to 318 mm. In comparing the conception period on the Boso Peninsula with that of other populations (Fig. 2), it was found to be one month earlier than in Hokkaido, which is 10 degrees of latitude north of the Boso Peninsula (Suzuki *et al.* 1996), and was about 10 days earlier than in Hyogo Prefecture (35°N, Koizumi 1991).

The breeding season is later at more northerly latitudes in mountain sheep (Bunnell 1982) and in reindeer (Leader-Williams 1988), because it is related to phenological differences in dietary vegetation (Bunnell 1982). In reindeer populations, calving occurs one month earlier per 10 degrees higher latitude (Leader-Williams 1988), a relationship which is supported by our own study of Sika deer. The leaves of deciduous trees on the Boso Peninsula, common
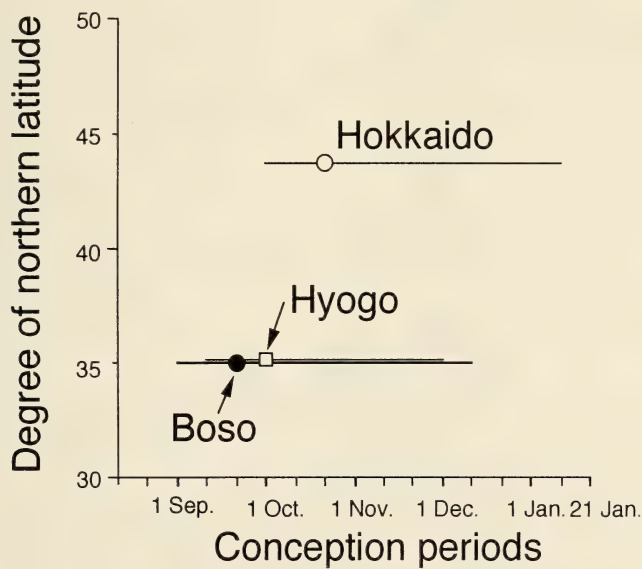

Fig. 2 Conception periods of Sika deer in Hokkaido, Hyogo and Boso. Bars show periods, and open circle, rectangle and solid circle indicates peaks of conceptions in Hokkaido, Hyogo and Boso, respectively. Data for Hokkaido and Hyogo are from Suzuki *et al.* (1996) and Koizumi (1991), respectively.

browse of the deer, begin to develop from early April to early May whereas in Hokkaido they develop from early May to mid May (Watanabe 1978, Sasaki 1983).

On the Boso Peninsula, local differences in the frequency distribution of conception were recognized from late October onwards (Fig. 3).   During this period, pregnancy ratios were 16.7% in the KG, 19.2% in the KU, and 26.5% in the OT sub-areas, though only two deer (3.2%) were pregnant in sub-area AT, where deer density was high, and in sub-area KT, this tendency was not clear because of the small sample size.

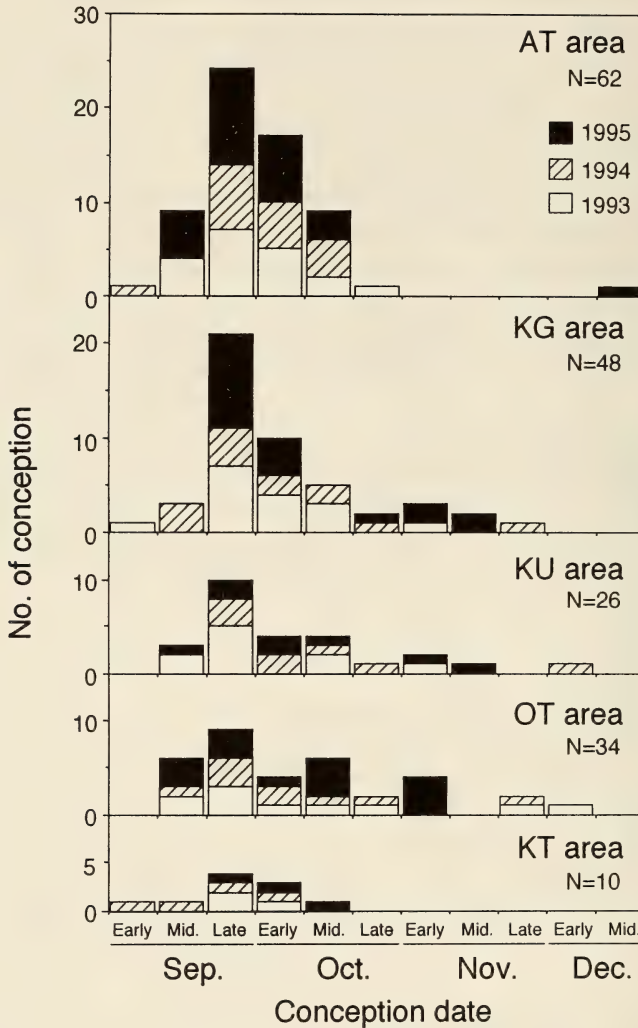In Nara Park, tame Sika deer at a high population density (276 / km²)

Fig. 3   Estimated distribution of conception date of Sika deer on the Boso Peninsula, central Japan.   Samples were collected in January and February 1993, and February and March 1994 and 1995.

conceived synchronously (Miura 1984). Koizumi (1991) thought that such synchrony of conception was a consequence of gregariousness, a factor which also appears to be born out by our own observations from the Boso Peninsula.

Among Cervid deer, it is known that the conception rate, in any particular age class, is related to body weight during the rutting season. Thus, only deer above a specific body weight threshold can conceive (Mueller and Sadleir 1979, Hamilton and Blaxter 1980, Verme and Ullrey 1984, Sadleir 1987, Langbein and Putman 1992). Young deer conceive later than older deer, because they achieve this weight threshold later (Smith 1974, Hamilton and Blaxter 1980, Suzuki *et al*. 1996). To examine the relationship between the age of pregnant females and conception date, maternal age classes and conception periods were compared (see Table 1). Although four-year-old and older deer tended to conceive earlier than did younger deer, no significant difference was detected (χ^2-test; $p > 0.05$), *i. e.* the conception period appeared to be independent of maternal age on the Boso Peninsula. In Hokkaido, during the second half of the conception period, only 4% of two-years-old or older females were pregnant (Suzuki *et al*. 1996), whereas on the Boso Peninsula 13% of such young females from all five sub-areas, and 18% from four sub-areas, excluding the high density AT sub-area, were pregnant. Thus, it appears that conception among the two-year-old and older females is less synchronized on the Boso Peninsula than it is in Hokkaido. Since deer densities on the Boso Peninsula (with the exception of sub-area AT) and in Hokkaido were similar, at 5.0 ± 4.9 (mean$\pm$ SD/km² n=10, Chiba Prefecture and Deer Research Group on Boso 1993) and 4.6 ± 4.9 (n=21, Hokkaido Institute of Environmental Sciences 1995), respectively, it is considered that this regional difference in conception synchrony was not due to differences of density.

We believe that this difference results from variation in the phenology of food plants used by the deer in different regions. It has been considered that the optimum periods for conception and parturition are affected by the periods of peak growth of the available vegetation (Bronson 1989). Bunnell (1982) showed that mountain sheep at more northerly latitudes began lambing later and lambed over a shorter duration than did sheep at more southerly latitudes, and that the timing of lambing was determined primarily by forage quality and quantity.

As mentioned above, spring leaf growth occurs approximately one month earlier on the Boso Peninsula than in Hokkaido. In Hokkaido, deciduous trees change color in autumn from late September onwards (Sasaki 1983), whereas they do so from mid-October onwards on the Boso Peninsula (Watanabe 1978). Furthermore, the first snows of winter occur from November onwards in Hokkaido, whereas little snow falls at all on the Boso Peninsula. Sika deer on the Boso Peninsula can continue to eat evergreen leaves from fall to winter (Asada and Ochiai 1996). Therefore, the duration of the optimum period for parturition seems to be longer, and synchrony seems to be weaker on the Boso Peninsula than in Hokkaido.

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Short Communication

The author and date of publication of the Sikkim vole *Microtus sikimensis*

Yukibumi Kaneko and Chris Smeenk[1]

Biological Laboratory, Faculty of Education, Kagawa University, Takamatsu 760, Japan
Fax. 0878-36-1652.
[1]*National Museum of Natural History, P. O. Box 9517, 2300 RA Leiden, The Netherlands*

The scientific name of the Sikkim vole has variously been given as *Neodon sikimensis* Hodgson, 1849 (see Jerdon 1874, Miller 1896 but misspelled *sikkimensis*, Palmer 1904, Hinton 1926 without the date of publication, Ellerman 1941) ; *Arvicola sikimensis* (Hodgson, 1849) (see Sclater 1891) ; *Pitymys sikimensis* (Hodgson, 1849) (see Ellerman 1947, Ellerman and Morrison-Scott 1951, Ellerman 1961, Frick 1968, Weigel 1969, Abe 1971, Mitchell 1975, Qian and Feng 1974, Corbet 1978, Honacki *et al.* 1982, Feng *et al.* 1984, 1986 ; misspelled *sikkimensis* by Ellerman 1947 and Frick 1968) ; and *Microtus sikimensis* (Hodgson, 1849) (see Gromov and Polyakov 1977, Sokolov 1988, Tan 1992, Musser and Carleton 1993, misspelled *sikkimensis* by Sokolov ; Sokolov and Tan without the date of publication). Thus, it is generally accepted that the author and date of publication are Hodgson, 1849.

The paper of 1849 referred to by these authors was published as a letter to Richard Taylor, one of the editors of "The Annals and Magazine of Natural History, London". This letter, however, was written not by Hodgson, but by Thomas Horsfield. Moreover, in this note the species was not formally described. On this vole Horsfield (1849) wrote as follows (p. 203) :

"5. NEODON, n. g., Hodgson.

Neodon Sikimensis Hodgs. This animal Mr. Hodgson considers as a new type, though in many respects allied to *Arvicola*. Mr. J. E. Gray at my request has kindly compared the specimen with the Murines from India contained in the British Museum ; it appears to be nearly allied to *Arvicola Roylei*, Gray, described in the "Annals of Natural History", vol. x. p. 265. There are, however, in the *Neodon* some differences in the folds of the upper and lower grinders ; these, with the other distinguishing characters of this type, will be pointed out in Mr. Hodgson's detailed description".

The name, therefore, is a *nomen nudum* here, apparently a manuscript name used by the collector, B. H. Hodgson.

Hodgson's expected description never appeared. Two years after his first announcement, Horsfield (1851 : 145-146) formally described the genus *Neodon*, based on the structure of the teeth, and the species *sikimensis*, giving characteristics of the pelage and some external measurements. Here as well as else-

where (Horsfield 1856 : 401), he mentions Hodgson as the author for the species. Accordingly, Blyth (1863 : 125) and Jerdon (1874 : 217) used *Neodon sikimensis* Hodgson (misspelled *sikhimensis* by Blyth).

Blanford (1879 : 41–42) already noted that the generic and specific names of this vole (misspelled *sikkimensis* here) had not been proposed by Hodgson, but by Horsfield (1849), though not accompanied by a description necessary to validate the names. Overlooking the description by Horsfield (1851), he remarked that, because of the lack of a description by Hodgson, Jerdon (1874) appeared to be the first author who had definitely described the species. Later, however, Blanford (1891 : 433) used *Microtus sikimensis*, referring to "*Neodon sikimensis* Hodgson, Horsfield, A. M. N. H.(2)iii, p. 203 (1849) (no description)". Wroughton (1920) added to the confusion by giving *Microtus sikimensis* Hodgson and *M.* (*M.*) *sikimensis* Horsfield on the same page, without further comment. In spite of the fact that all later authors (see above) have Hodgson, 1949 as the author and date of publication for *sikimensis*, it is evident that the first valid description of *Neodon sikimensis* was published by Horsfield (1851).

The type specimen (BM 79. 11. 21. 395) is in the British Museum (Natural History)=now Natural History Museum, London. The original label reads (front) : "*Type* of Neodon sikimensis Horsf., Loc. Sikim, Ex. Coll. Hodgson", and (back) : "*Type* of Neodon sikimensis Horsf. *No skull*". In the mammal catalogue of the National Museum of Natural History, Leiden (Jentink 1888 : 89), there is one mounted skin of *Arvicola sikimensis* Hodgson (a ; present catalogue number RMNH 19144), collected by Hodgson in Tibet and given as one of the types of the species. From Horsfield's note and description, however, it is clear that the author had only one animal before him at the time of these publications. In 1849 he wrote about "the specimen" ; in 1851 too, he mentioned only one specimen : "A. Presented by B. H. Hodgson, Esq.", his measurements are of one animal, and he gave "Sikim" as the place of origin. The material collected by Hodgson and now in the Leiden Museum was received in 1853. It was presented by Horsfield with a letter to the museum dated 15 November 1853, in which he writes that the specimens had been collected by Hodgson in Tibet and Nepal. Therefore, it is obvious that the skin of *Neodon sikimensis* included with this material and specified in Horsfield's letter, was received by Horsfield after the species had been described. Consequently, the Leiden skin is not a type. Specimen BM 79.11.21. 395 (incorrectly quoted as BM 79.11.21.397 by Wroughton 1920) is the holotype of *Neodon sikimensis* Horsfield, 1851.

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THE MAMMALOGICAL SOCIETY OF JAPAN

The Mammalogical Society of Japan publishes original papers in two journals : the *Mammal Study* (the continuation of the *Journal of the Mammalogical Society of Japan*) for papers written in English, and *Honyurui Kagaku* [Mammalian Science] for those submitted in Japanese. Each journal is published twice a year. Submissions are considered on the understanding that they are being offered solely for publication by the Mammalogical Society of Japan. In principle, authors submitting articles to the journals should be members of the Mammalogical Society of Japan. Both journals are distributed free of charge to the members of the Society.

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FOREWORD

After long and distinguished careers in the fields of mammalian biology and ecology, two renowned scientists retired in March 1997 : Dr Hisashi Abe, former professor of the Faculty of Agriculture at Hokkaido University, and Dr Satoshi Shiraishi, former professor of the Faculty of Agriculture at Kyushu University.

Between them, they have served as presidents of the Mammalogical Society of Japan since 1991. The Mammalogical Society of Japan owes both professors a great debt of gratitude for the considerable efforts that they have made in stimulating the development of, and the activities of, the Society.

Dr Hisashi Abe

Dr Abe obtained his scientific education from Hokkaido University, he continued to do research there and, ultimately, through his teaching and research career there, he has contributed influentially to the education of thousands of younger scientists.

He received his Bachelor of Agriculture degree from Hokkaido University's Laboratory of Applied Zoology in 1956. He then continued with graduate studies on small mammals, also at Hokkaido University, earning his doctorate in Agriculture under Professor Tetsuo Inukai for his studies on the classification and biology of the Japanese Insectivora (Abe 1967, 1968).

Dr Abe was appointed to his first academic position at Hokkaido University's Natural History Museum in 1961, where he worked for eight years. Then, in 1969, he became an associate professor in the Laboratory of Applied Zoology at Hokkaido University, where he was appointed professor in 1992.

Dr Abe was born and grew up until graduating from high school in Tokushima Prefecture in western Japan. In his mountainous home town he developed a fascination for living organisms, and for collecting and preserving specimens, all of which are now held at the Tokushima Prefectural Museum. Such childhood experiences formed the basis for his later research career, during which he has collected over 7,000 specimens of mammals, particularly of insectivores and rodents, which are preserved at the Natural History Museum, Faculty of Agriculture, Hokkaido University.

His main area of interest has been the biogeographical question of why certain species occur in certain places, a question which he has examined from the perspectives of phylogenetic relationships, inter-specific interactions and habitat structure. He first described and discussed the phylogenetic and ecological relationships among Japanese insectivores based on their morphology and their life histories in the 1960s, returning again to the subject for further publication in the 1990s (Abe 1967, 1968, 1996), he analyzed the community structure of insectivores and rodents in Nepal and Japan using an index of

morphological overlap between species (Abe 1982), and he also described, with Dr Shiraishi, a new species of mole, *Nesoscaptor uchidai* (Abe *et al.* 1991). He has published numerous papers based on his own collections, but perhaps his popular publication has been "A Pictorial Guide to the Mammals of Japan" (Abe *et al.* 1994), a distinguished book, well illustrated, which provides much new information on Japanese mammals, and which he edited and authored.

In addition to his own research studies, Dr Abe has introduced innumerable students to various aspects of ecology and ecological methodology. He has directed various masters degree students and supervised a number of doctoral dissertations in the fields of applied zoology, ecology and taxonomy.

He has recently carried out molecular phylogenetic studies of insectivores with young co-workers using his specimens, with the latest techniques confirming the conclusions he had reached in his previous studies (*e.g.*, Ohdachi *et al.* 1996, Okamoto and Abe in prep.).

Dr Satoshi Shiraishi

Dr Shiraishi graduated from the Faculty of Agriculture of Kyushu University in 1958. He completed his doctoral degree and was appointed as an assistant researcher at the Faculty of Medical Science of Kurume University. From 1967, he worked at the Forestry Station of the Ministry of Agriculture and Forestry of Japan, but then he returned to the Faculty of Agriculture at Kyushu University as an associate professor in 1974 and was appointed professor there in 1990.

His studies have been very wide-ranging, including the taxonomy of rodents and other small mammals (Okura *et al.* 1984, Ando *et al.* 1990), the morphology and ecology of birds, the ecology and functional morphology of ticks, and the biology of parasites. He has studied the ecology of flying squirrels, *Petaurista leucogenys* (Ando *et al.* 1985), and of more than ten species of mice, particularly the biology of their growth (Lin *et al.* 1993, Yoshinaga *et al.* 1997). Of special significance was his discovery of not just a new species but a new genera of mole, *Nesoscaptor uchidai*, on the Senkaku Islands, southern Japan (Abe *et al.* 1991), a discovery as exciting as that of the discovery of the Iriomote cat, *Felis iriomotensis*. Dr Shiraishi also studied the ecology and reproduction of hares, *Lepus brachyurus* (Yamada *et al.* 1990) and the growth of the Japanese weasel, *Mustela itatsi*.

Dr Shiraishi has not only worked as a mammalogist, in the field of ornithology, he studied the Eastern great white egret, *Egretta alba modesta* (Min *et al.* 1984) and the black kite, *Milvus migrans* (Koga *et al.* 1994). His study of egrets was instrumental in their promotion as a specially protected species, while his kite studies were directed towards the reducton of air traffic accidents involving birds.

Furthermore, in the field of parasitiolgy, he studied the reproduction, ecology, functional morphology and physiology of the cattle tick, *Haemaphysalis longicornis* (Kakuda *et al.* 1992), which transports *Taireria sergenti*

(protozoa) and causes taireriosis in calves, and established a control system using microscopic, physiological and histochemical techniques. He first studied *Schistosoma japonicum* at Kurume University, and continued his parasitalogical studies at Kyushu University, during which he discovered a new species, *Tikusnema javaense*, in a rodent from Indonesia (Hasegawa *et al.* 1992).

He made great efforts to introduce the study of the ecology of Australian animals to Japanese scientists, students and the public, and was honored for his efforts in this area by the Australian government in 1986 by being made the recipient of the fifth Southerncross Prize. He also worked in Indonesia in 1980/81 as a specialist consultant for JICA in the field of rodent pest control.

Dr Shiraishi has contributed greatly in the fields of mammalogy, ornithology and parasitology. He has made considerable contributions to both university and academic societies, including serving as president of the Mammalogical Society of Japan from 1995 to 1996, and has influenced and educated innumerable students with his wide knowledge and gentle personality.

In appreciation of Dr Abe's and Dr Shiraishi's work in the field of mammalogy, and for their tremendous contribution to the society, The Mammalogical Society of Japan decided at its 1996 annual meeting held at Kyushu University to publish a special issue of "Mammal Study" (the continuation of the "Journal of the Mammalogical Society of Japan") commemorating their retirement.

Following this decision, the editorial board of "Mammal Study" requested members to submit memorial papers. The committee also asked two members, Dr T. Saitoh and Dr T. Mori to join the editorial team for this special issue. Six special papers, in addition to four other research papers, were subsequently accepted for publication. The committee deeply appreciates the work of these contributors and the two supporting editors.

The committee and all the members of the Mammalogical Society of Japan express their hearty congratulations to Dr Hisashi Abe and Dr Satoshi Shiraishi on their retirement, and celebrate the importance of their scientific work in the publication of this special issue of "Mammal Study". We hope and trust that Dr Abe and Dr Shiraishi, though retiring from their university positions, will, however, continue in encouraging and guiding the work of younger generations of scientists for many more years to come.

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Seiki TAKATSUKI (Editor-in-chief), Takashi SAITOH and Takanori MORI

Cross-species amplification of microsatellite DNA in Old World microtine rodents with PCR primers for the gray-sided vole, *Clethrionomys rufocanus*

Yasuyuki Ishibashi[1], Takashi Saitoh[2], Syuiti Abe[1,3], and Michihiro C. Yoshida[1,3]

[1] *Chromosome Research Unit, Faculty of Science, Hokkaido University, North 10, West 8, Kita-ku, Sapporo 060, Japan*
Fax. +81-11-736-6304, e-mail. stone@ees. hokudai. ac. jp
[2] *Wildlife Management Laboratory, Hokkaido Research Center, Forestry and Forest Products Research Institute, Hitsujigaoka 7, Toyohira-ku, Sapporo 062, Japan*
[3] *Laboratory of Cytogenetics, Graduate School of Environmental Earth Science, Hokkaido University, North 10, West 5, Kita-ku, Sapporo 060, Japan*

Abstract. Applicability of seven primer sets, originally designed for polymerase-chain-reaction (PCR) amplification of microsatellite DNA in the gray-sided vole, *Clethrionomys rufocanus*, was examined in other 12 microtine species from three genera (*Clethrionomys*, *Eothenomys* and *Microtus*). Of the primer sets used, one distinctly amplified PCR products in all the species examined. Three sets gave PCR products in all but one species. The remaining three sets failed to amplify any products in several species. Non-amplification occurred mostly in *Microtus* species, although two primer sets were not available for two *Clethrionomys* species. Since most amplified loci showed allelic variations, the present primers are useful for molecular ecological studies of related microtines, especially *Clethrionomys* and *Eothenomys* species.

Key words. *Clethrionomys*, *Eothenomys*, microsatellites, *Microtus*, PCR primer.

Microsatellite loci, which consist of tandem repeats of short DNA sequence motif (≦5 base-pairs), are highly variable in repeat number, thereby providing an excellent molecular marker for both ecological and population genetic studies (Burke *et al*. 1992, Queller *et al*. 1993). Genotyping at microsatellite loci facilitates assessment of paternity (Morin *et al*. 1994b, Sillero-Zubiri *et al*. 1996) or relatedness (McDonald and Potts 1994, Blouin *et al*. 1996, Ishibashi *et al*. 1997), and also allows to summarize the genetic structure within or among populations (Morin *et al*. 1994a, Paetkau *et al*. 1995, Lade *et al*. 1996).

Microsatellites can be amplified from a minute amount of DNA using the polymerase chain reaction (PCR) technique (Litt and Luty 1989, Tautz 1989, Weber and May 1989). Hair roots (Washio 1992, Morin *et al*. 1994a), bones (Taberlet and Fumagalli 1996) or feces (Tikel *et al*. 1996) can all be used as sources of DNA, if necessary. PCR-based analysis has a great advantage over conventional allozyme analysis, because of the high resolution and because

sample collection is none, or less, invasive.

Microsatelites are thought to be localized in rapidly evolving non-coding regions, and hence cross amplification is generally restricted to closely related species (Schlötterer *et al*. 1991, Coltman *et al*. 1996, Kayser *et al*. 1996, Valsecchi and Amos 1996).   In this study, a cross-species microsatellite amplification was conducted in 12 species of Old World microtine rodents.   Seven primer sets originally designed for the gray-sided vole, *Clethrionomys rufocanus*, in Hokkaido, Japan, were used.   So far microsatellite loci have not been cloned in other microtines, thus cross amplification could justify the applicability of these microsatellite primers in the species examined.

MATERIALS AND METHODS

Seven microsatellite primer sets, designed for *Clethrionomys rufocanus* in Japan, were used in this study (Table 1).   They consisted of primer sets for five loci previously cloned, MSCRBs-1 to -5 (Ishibashi *et al*. 1995), and two further loci, MSCRBs-6 and 7, newly cloned from the *C. rufocanus* genomic library and sequenced as described by Ishibashi *et al*. (1995).   One of the paired primers was newly designed for two loci, MSCRBs-2 and -5, so as to shorten the size of PCR product.   For MSCRB-3, one of the paired primers was also redesigned so as to avoid non-amplification which is caused by base substitutions near the CA- and GA-repeats (Ishibashi *et al*. 1996).   Cross-species amplification was performed using one to six individuals from each of 12 species from the three genera, *Clethrionomys*, *Eothenomys* and *Microtus* (Table 2).   Three *Clethrionomys* species captured in three widely different localities, Japan, Finland

Table 1.   Microsatellite primer sets used in this study, including those for the previously described loci, MSCRBs-1 to 5 (Ishibashi *et al*. 1995) and newly cloned loci, MSCRBs-6 and -7.

Locus	Repeat structure[a]	Primers (5'-3')	TA[b]	Product size[c]
MSCRB-1	$(AC)_{24}$	AGTGTTTGGAAGCCATGCGGTA CAGGAGCTTCATGGCTGGAATA	58	150-270
MSCRB-2	$(AC)_{23}$ with several short AC-repeats	AAGGGTGAGTATGCCAATCA TCTCAGATTCTGTGATATGCTGTC[d]	48	100-200
MSCRB-3	$(CA)_{19}(GA)_{24}$	CATGACCTTCTATTTCTGTCAG CTCTAGCATGATGTTACTGT[d]	48	250-350
MSCRB-4	$(CA)_{20}$	GTGCTGCTTACTGGCTTCTTGT CCTGAGTTGTATAAGAAAGCAGGC	60	70-130
MSCRB-5	a mixture of CA-, ATAC- and ATGT-repeats	GGTTGGTGTTTGCATTTAGG CGTCTGGGTTTTACATCTGA[d]	54	130-230
MSCRB-6	$(AC)_{12}(AG)_{25}$	TATAATAGATTTGAGTATCTGC GATGTCCATCAAGTTAATCGT	52	150-220
MSCRB-7	$(AC)_{20}$	GTTTTATGTTAGTCTCATCTG AGGCAATCCTGGTGAGTAACA	52	80-150

[a]Nucleotide sequence of the clones obtained from the Japanese *C. rufocanus* genomic DNA library, [b]Annealing temperature in PCR (°C), [c]Estimated PCR product size for all the species examined in this study (in base-pairs), [d]The primer sequence differed from that previously described by Ishibashi *et al*. (1995).

Table 2. Cross-species amplification with seven pairs of *C. rufocanus* microsatellite primers.

Genus	Species[a]	Location	N[b]	Locus[c]						
				MSCRB-1	MSCRB-2	MSCRB-3	MSCRB-4	MSCRB-5	MSCRB-6	MSCRB-7
Clethrionomys	C. rufocanus	Japan	>5	H/+	H/+	H/+	H/+	H/+	H/+	H/+
		Finland	5	H/+	H/+	H/+	H/+	H/−[d]	H/−	H/+
		Norway[e]	5	H/+	H/+	H/+	H/+	H/−[d]	H/+	H/+
	C. glareolus	Finland	5	0	H/+	H/+	H/+	L/+	H/+	0
		Norway[e]	5	L/+	H/−	H/+	H/+	L/+	H/+	0
	C. rutilus	Japan[e]	5	0	H/+	H/+	H/+	H/−	H/+	0
		Finland	5	L/−	H/+	H/−	H/+	H/+	H/−	0
		Norway[e]	5	L/−	H/+	H/−	H/+	H/+	H/−	0
	C. rex	Japan	5	H/+	H/+	H/+	H/+	H/+	H/+	H/+
Eothenomys	E. andersoni	Japan (Tohoku)	5	H/+	H/+	H/+	H/+	H/+	H/+	H/+
		Japan (Kii Pen.)	3	L/+	H/+	L/+	H/+	H/+	H/+	L/+
	E. smithii	Japan	5	H/+	H/+	H/+	H/+	H/+	H/+	H/+
	E. regulus	Korea	5	H/−	H/+	H/+	L/−	H/+	H/+	H/+
Microtus	M. montebelli	Japan	6	0	H/+	0	H/+	H/+	H/+	L/−
	M. arvalis	The Netherlands	1	0	H/+	L/+	0	H/+	0	H/+
	M. kikuchii	Taiwan	1	0	H/+	0	H/+	L/−	H/+	H/−
	M. melanogaster	Taiwan	2	L/−	L/+	L/−	H/+	H/+	H/+	0
	M. nivalis	Swiss	1	H/−	0	0	H/−	H/−	H/−	H/+
	M. oeconomus	Norway[e]	4	0	H/+	0	H/+	H/−	H/+	H/−

[a]Species names follow Abe *et al.* (1994) for Japanese species and Corbet and Hill (1991) for all others, [b]Number of individuals analyzed, [c]Abbreviations ; 0 : not amplified (no product or smear), H : amplified at higher annealing temperature, TA (see Table 1), L : amplified at lower annealing temperature, TA−10, − : monomorphic, + : polymorphic, [d]Non-amplification in some individuals (see text for detail), [e]Laboratory-bred animals.

and Norway, were also examined for a possible variation in the applicability of these microsatellite primers (Table 2).   DNA was isolated from each animal using the conventional phenol/chloroform method (Sambrook *et al*. 1989).

The PCR amplification was carried out in 10 μl of reaction mixture containing 50 mM of KCl, 1.5 mM of MgCl$_2$, 10 mM of Tris-HCl (pH 8.3), 0.2 mM of dNTP, 0.25 μM of each primer, and 0.25 unit of *Taq* DNA polymerase (TaKaRa).   About 30 ng of genomic DNA was used for each reaction.   After denaturation at 93°C for two minutes, the reaction was carried out for 30 cycles under the following conditions using a DNA Thermal Cycler PJ2000 (Perkin Elmer Cetus) ; 93°C for 30 sec, TA°C (see Table 1) for 20 sec, and 72°C for 20 sec. TA of each primer was optimized to amplify apparent PCR products in Japanese *C. rufocanus* after calculating with the formula : 69.3+0.41×(% of GC content)−650/(primer length) (Mazars *et al*. 1991).   When amplification failed in species other than Japanese *C. rufocanus*, lower annealing temperature by 10°C, *i.e.*, TA−10, was adopted so as to allow for mismatches in the primer sequence in the subsequent trials.

The PCR products were electrophoresed in a 3% agarose gel and an 8% non-denatured polyacrylamide gel in order to examine the results of amplification and allelic variation.   When amplification in a species did not result in any products, or showed only a smearing pattern, under the above PCR conditions, the result was categorized as "not amplified".   If all individuals examined showed a single band only, such a species was categorized as "monomorphic". If two bands of similar size and amount were apparent in one or more individuals, then the species was categorized as "polymorphic".

RESULTS AND DISCUSSION

Of seven microsatellite primer sets used, one provided apparent PCR products in all twelve species examined (MSCRB-5, Table 2).   Three sets (MSCRBs-2, -4 and -6) gave products in all but one species.   The remaining sets (MSCRBs-1, 3 and 7) failed to amplify any products in several species (see Table 2).   When amplification was performed with the primer set for MSCRB-3 under the lower annealing temperature, ladder-like band patterns were observed from low to high molecular weight regions.   Despite the many spurious bands, we categorized them as "amplified" if the ladder included an apparent band(s) of the molecular size similar to other microtines' products. Non-amplification occurred mostly in *Microtus*, although no apparent product was amplified with the MSCRBs-1 and -7 primers in either *C. rutilus* or *C. glareolus*.   In all *Eothenomys* species, products were obtained from all seven primer sets under higher or lower annealing temperature (Table 2).

Non-amplification of microsatellite loci may occur as a result of nucleotide sequence variation (*e.g.*, base substitution, deletion and/or addition) within the priming site for PCR amplification.   Therefore, the observed non-amplifications could be due to variation within the priming sequences. Furthermore, in the present study, no PCR products were observed in five of ten

Scandinavian *C. rufocanus* at MSCRB-5 (Table 2). Since allelic variation at the locus is very small in Japanese *C. rufocanus* (Ishibashi *et al.* 1995), these five individuals may be homozygous for a non-amplifying (null) allele with sequence variations in the priming site. Although such null alleles were not detected in microtines oher than the Scandinavian *C. rufocanus*, it is clearly important to pay attention to the possible presence of null alleles especially when using heterologous microsatellite primers (Paetkau and Strobeck 1995, Pemberton *et al.* 1995).

Despite the allelic variation in most amplified loci in each species, interpretation must be made with some caution. In the present study, "polymorphic" and "monomorphic" species are arbitrarily defined on the basis of the number of alleles (bands) in the limited number of DNA samples examined (Table 2). For *C. rufocanus*, *C. glareolus*, *C. rutilus* and *M. oeconomus* from Norway, and *C. rutilus* from Japan, the DNA samples used were extracted from laboratory-bred individuals (Table 2). These animals might have lost heterozygosities at some loci by chance during laboratory breeding. The observed monomorphic band patterns at several loci may not, therefore, indicate the real situation in natural populations.

The present study, though preliminary in nature, demonstrates that most PCR primer sets for *C. rufocanus* microsatellites are useful for detecting allelic variations in related microtines, especially in *Clethrionomys* and *Eothenomys* species. Given the small sample size and the non-systematic collection, further examinations are required to clarify the presence of null alleles and of allelic variation in each population or species of interest.

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Laboratory experiments on spatial use and aggression in three sympatric species of shrew in Hokkaido, Japan

Satoshi OHDACHI

Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan
Fax. +81-11-706-7142, e-mail. ohdachi@bio. hokudai. ac. jp

Abstract. Aggression and the use of vertical and horizontal space in the presence of con- or hetero-specific individuals were investigated in laboratory for *Sorex unguiculatus*, *S. caecutiens*, and *S. gracillimus* in Hokkaido, Japan. *S. unguiculatus* frequently used the below floor strata of experimental cages or subterranean burrows as their main area of activity, whereas *S. caecutiens* and *S. gracillimus* mainly used the cage floor or the ground surface. The presence of con- or hetero-specific individuals led to no changes in any of the three species in the use of space, or in behavioral patterns (active/inactive ; underground/resting/ moving on the ground surface). When two individual shrews were introduced into two interconnected cages, they tended to remain in separate cages, with the exception of *S. gracillimus* with a conspecific. Dominance rank was highest in *S. unguiculatus*, intermediate in *S. caecutiens*, and lowest in *S. gracillimus*. *S. caecutiens* attacked *S. gracillimus* most frequently and *S. gracillimus* received attacks from *S. caecutiens* most frequently. The implication of this research is that severe interference competition may occur in the field between *S. caecutiens* and *S. gracillimus*.

Key words : coexistence, interference competition, niche shift, surface activity, underground activity.

Sorex unguiculatus, *S. caecutiens*, and *S. gracillimus* are three common species of shrew occurring throughout Hokkaido. When *S. caecutiens* and *S. gracillimus* occur together, they are never the two most abundant species (Ohdachi and Maekawa 1990a, Ohdachi 1995a). Ohdachi (1995b) confirmed that *S. caecutiens* and *S. gracillimus* share a greater similarity in their diets than do either of these species and *S. unguiculatus*. These findings indicate that interspecific competition is likely to be more severe between *S. caecutiens* and *S. gracillimus*. Further, *S. unguiculatus* is a much stronger burrower than either of the other two species (Ohdachi 1995c). It is suspected, therefore, that severe interference for space exists between *S. caecutiens* and *S. gracillimus*.

There is the potential for a niche shift by one species, when in the presence of the other, that could influence the outcome of competition. If both species exhibit interference competition, but neither of them changes any of its niche

dimensions, then the physically superior individual or species may exclude the inferior individual or species from good habitat or a good position (*e.g.*, Hardin 1960, Schoener 1975, Werner and Hall 1976, Holbrook 1979, Parker and Suther-land 1986, Alatalo and Moreno 1987, Arthur 1987). In such cases, aggressive behavior and physical superiority are essential keys for guild formation, and thus make it interesting to investigate whether individuals change their use of space (or niche) in the presence of other individuals.

For cryptic species whose life histories are poorly known, such as the shrews of Hokkaido, it is difficult to carry out extensive field studies of space use and interactions. Ohdachi (1992) described the home ranges of sympatric shrews in Hokkaido, but was only able to present limited information about interspecific interactions because of the difficulties in observing them directly. Therefore, the alternative means of investigating direct interactions in the laboratory was chosen for this study. Although the reality of simulated situations, particularly in the scaling of time and space, is questionable (Bennett 1990), the results obtained from laboratory experiments can, nevertheless, complement those from field studies (Diamond 1986, Hairston 1989, Keddy 1989).

This paper serves to describe : (1) interspecific differences in the use of space, (2) interspecific interactions such as aggressive behavior, and (3) the impact of the presence of another individual on the use of activity space and on behavioral patterns, in *S. unguiculatus*, *S. caecutiens*, and *S. gracillimus* in Hokkaido. For these purposes, two different laboratory experiments were conducted.

MATERIALS AND METHODS

1. Experiment 1

The first experiment was designed mainly to examine the effects of the presence of con- or hetero-specific individuals on vertical space use. Animals used in this experiment were nine *S. unguiculatus* (5 young males, 4 young females), eight *S. caecutiens* (1 adult male, 4 young males, 3 young females), and five *S. gracillimus* (3 young males, 2 young females), which were captured in Yufutsu Moor (Tomakomai-shi) during 14–18 June 1992 and in a wind-shelter belt near the Teshio Experimental Forest of Hokkaido University (Horonobe-cho) during 25 June to 27 August 1992. Basically, sexually immature individuals were used in experiments in order to lessen the potential effect of sexual behavior on space use. Shrews were kept under a 16-hr light and 8-hr dark photoperiodic cycle at $20 \pm 2°C$. The light intensity was maintained at 1420 lux during the light period and at 12 lux during the dark period (as measured at the center of laboratory floor ; See Ohdachi 1994, 1995c for details). Each experiment was conducted throughout the 8-hr dark period, from 11 October 1992 to 6 January 1993.

Each observation cage contained 20 levels and the floor surface, and was fitted with two staircases (Fig. 1). Each of the boards separating the levels
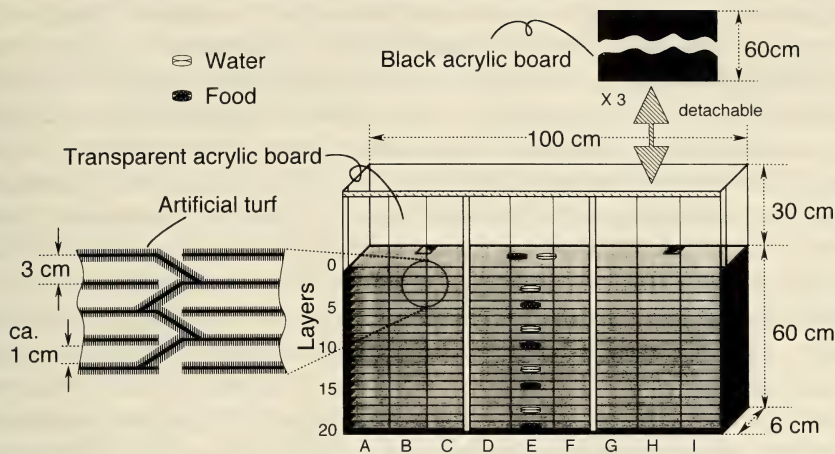
Fig. 1.   The experimental device for Experiment 1.   Black boards were removed just before an experimental session.

was covered on both sides with artificial turf so that shrews were always in physical contact with this surface while moving about between levels.   Pieces of tissue paper, which simulated ground litter, were located on the cage floor. Trays of the mixed paste diet and water were located as shown in Fig. 1. Black acrylic boards were attached in front of transparent cage walls, so as to exclude light before observation periods.

Either one or two animals were released simultaneously onto the cage floor thirty minutes before the onset of the dark period.   The black masking boards were gently removed immediately after the light was turned off.   The location and behavior of each shrew were then recorded every fifteen minutes using a weak red spot-light.   After finishing an experimental session, the animals were removed and the cages were washed with ethanol and kitchen detergent and then dried out.

The vertical location of a shrew was assigned to one of five categories: surface level (0), levels 1–5, 6–10, 11–15, and 16–20.   Utilization of each level by an individual was obtained by averaging the percent frequencies for the level among several experimental sessions under the same experimental treatment. Seventy experimental sessions were used for analysis.

The dominance relationship between two individuals was defined as follows: the "loser" was the individual which avoided, escaped, or fled from its "opponent" when two animals encountered or fought, while the opponent under these circumstances was a "winner".   If the number of wins and losses observed were the same, the two animals were judged to be "even".   When no direct contact was observed, this was defined as "no match".

2. Experiment 2

The second experiment was designed to investigate aggressive behavior

and the effect of the presence of con- or hetero-specific individuals on the use of space use (especially horizontal use) and behavioral patterns.   Animals used in this experiment included ten *S. unguiculatus* (1 adult female, 5 young males, 4 young females), three *S. caecutiens* (2 young male and 1 young female), and five *S. gracillimus* (2 adult females, 1 young male, and 2 young females), which were captured in wind-shelter belts near the Teshio Experimental Forest of Hokkaido University during 6-22 August 1993, and one adult female *S. caecutiens* that was captured in Yufutsu Moor in July 1992.   Laboratory conditions were the same as in Experiment 1.   Each experiment was conducted throughout the dark period, from 30 August to 24 November 1993.

Two animals were released separately into experimental cages (Fig. 2) one day before an experiment, with both sides of the connecting tube being closed by rubber plugs.   The rubber plugs were removed five minutes before the onset of the dark period.   As a control experiment, an empty cage was connected to a cage where a single shrew was introduced.   The first cage into which a shrew was introduced, prior to the cages being connected for the experiment, is hereafter referred to as the "home" cage, while the other is referred to as the "away" cage.

Shrew behavior was recorded using a video camera recorder (in the twilight vision mode) throughout the dark period, and sampled every 5 minutes while replaying the video tapes.   Behavior was ascribed to one of three categories : "underground activity" (shrews were underground or digging), "in
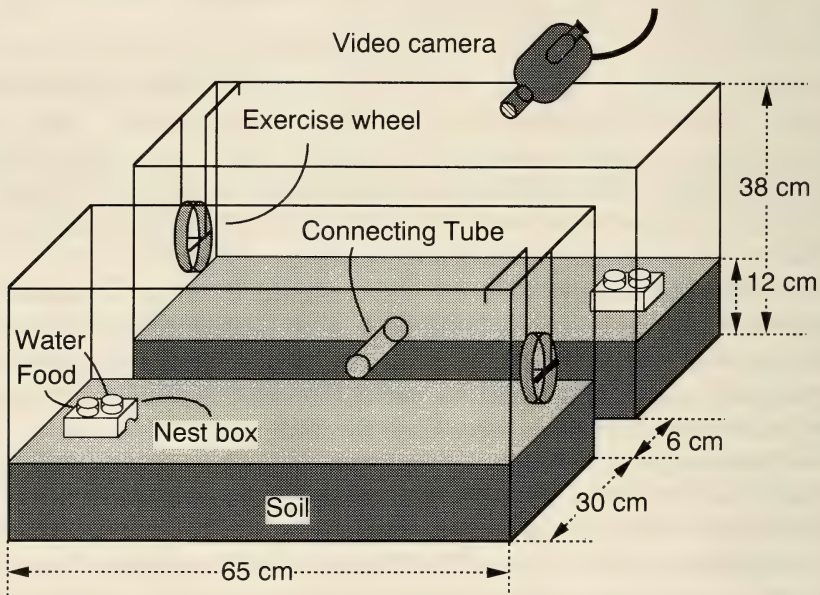

Fig. 2.   The experimental device for Experiment 2.   A connecting tube is plugged until an experimental session is started.   Note that the bottom of a nest box was open to the ground surface.

action on the ground" (shrews were in nest boxes or resting on the ground surface), and "moving on the ground" (shrews were walking or running on the ground surface, or whirling exercise wheels). Other behaviors, such as eating, drinking, or self-grooming, were usually too brief to be recorded by the 5-minute-interval sampling method. Behavior below ground and in nest boxes could not be observed in this experiment. Because *S. unguiculatus* usually constructed burrows in its "home" cage and some entrances of the burrows opened under its nest box, it was impossible to distinguish "underground activity" and "inaction on the ground" when it was in its nest box. According to preliminary observations, however, *S. unguiculatus* usually entered burrows under its nest box instead of staying on the ground surface when in its nest box. Therefore, unless it was possible to verify that the shrew did not enter a burrow, the case in which *S. unguiculatus* was in a nest box was classified as "underground activity". Preliminary observations revealed that *S. caecutiens* and *S. gracillimus* usually stayed on the ground surface under the nest box of *S. unguiculatus*, and that they were usually inactive there. Thus, when *S. caecutiens* or *S. gracillimus* was in the "away" nest box of *S. unguiculatus*, this was classified as "inaction on the ground", except when they obviously entered burrows under the nest box.

The frequency of each behavioral category for an individual was obtained by averaging the observation frequencies of the category across several experimental sessions under the same experimental treatment. Sixty-two experimental sessions (496-hour observation in total) were used for the analysis.

The number of attacks and the dominance relationship between individuals were determined by continuous scanning of the video tape throughout the 8-hr experimental session (complete observation). Attacking behavior includes chasing, biting body or tail, and wrestling. Attacks interrupted for more than 10 seconds was counted separately. The criteria for "win", "lose", and "no match" were the same as in Experiment 1. In this experiment, however, "even" was defined as follows: frequent counterattacks were observed or an individual did not escape from the opponent even when it was attacked often.

RESULTS

1. Experiment 1

Sorex unguiculatus was more subterrestrial than either *S. caecutiens* or *S. gracillimus*. *S. caecutiens* used the surface level significantly more frequently than *S. unguiculatus* during its active phase (ANOVA with arcsine transformation by Scheffe's method, $\alpha = 0.05$), but utilization of the other levels did not differ significantly between these two species (Fig. 3). *S. gracillimus* appeared to frequently use the surface level as did *S. caecutiens*, although its surface activity was not statistically different from that of either *S. unguiculatus* or *S. caecutiens* (Fig. 3).

Vertical space use did not differ significantly between the experimental treatments in each of the three species (Fig. 3). The dominance relation also
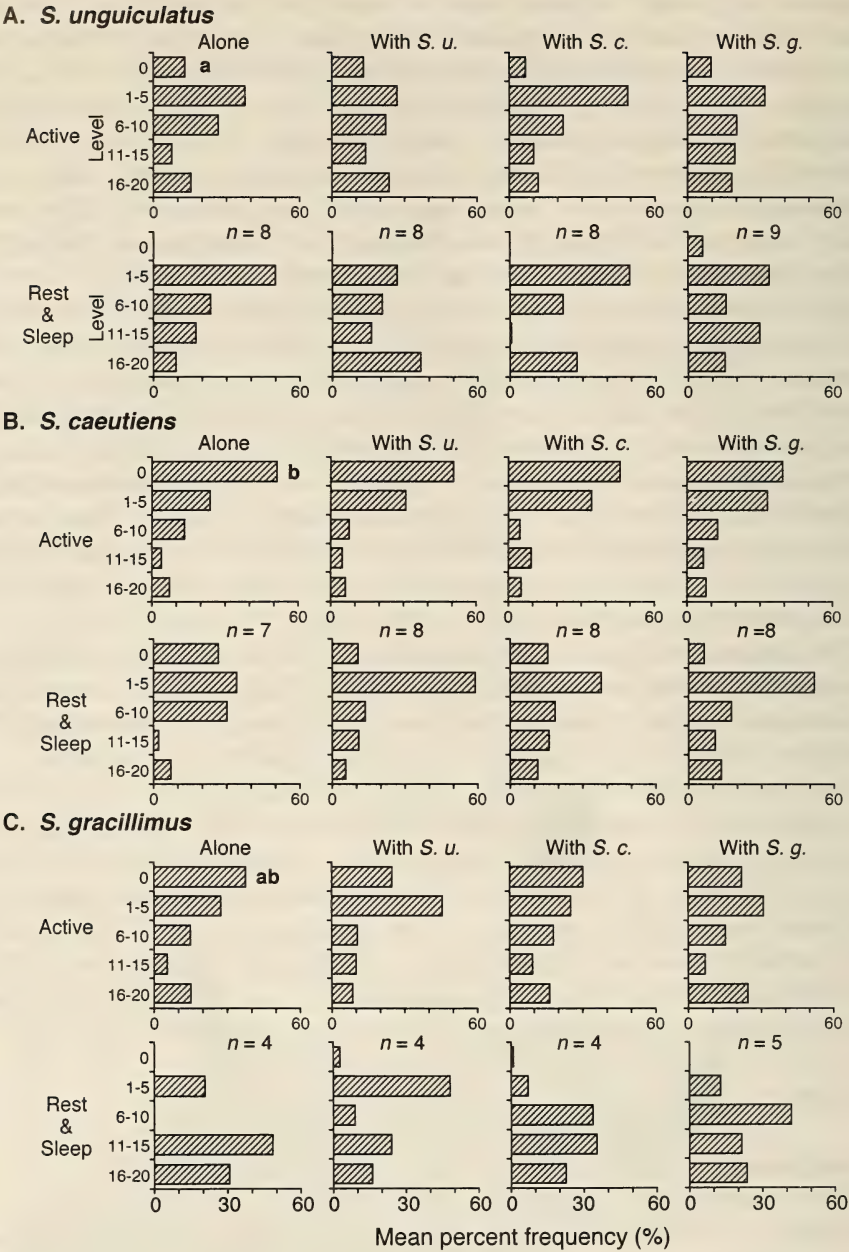
Fig. 3. Vertical spatial use of shrews when they were alone and with con- or hetero-specific individuals (mean percent frequency). The same bold letters (a, b) indicate non-significant difference in mean percent frequency for the floor surface (0) between species when shrews were "alone" ($\alpha=0.05$, ANOVA, arcsine transformation, Scheffe's method; the sequential Bonferroni correction among levels, Rice 1989). There was significant difference neither among species for the other levels when alone nor among experimental treatments for each level within species.

Table 1. The ratios of "active" and "rest & sleep" phases in three shrew species observed in Experiment 1 (mean percent frequency±*SD*). Mean percentages in the "alone" column differed significantly between any two of the three species (α=0.05, ANOVA, arcsine transformation, Scheffe's method). The different letters indicate significant differences. The mean percentages did not differ significantly among the experimntal treatments within species.

Experimental treatment	Alone	With		
		S. u.	*S. c.*	*S. g.*
S. unguiculatus	a			
Active	33.0±10.9	38.7±15.5	39.2±12.8	41.4±17.1
Rest and Sleep	67.0	61.3	60.8	58.6
(*n*)	(8)	(8)	(8)	(9)
S. caecutiens	b			
Active	70.6±12.1	73.3±17.9	65.5±25.0	68.6±8.2
Rest and Sleep	29.4	26.7	34.5	31.4
(*n*)	(7)	(8)	(8)	(8)
S. gracillimus	c			
Active	50.0±10.4	60.5±12.4	50.8±6.2	53.8±12.2
Rest and Sleep	50.0	39.5	49.2	44.2
(*n*)	(4)	(4)	(4)	(5)

had no apparent effect on vertical space use ; there were no significant differences for almost all comparisons.

The percentages of active and non-active phases did not differ significantly between the exrimental treatments (alone and with con- or hetero-specific individuals) in any of the three species (ANOVA with arcsine transformation by Scheffe's method, α=0.05, Table 1). Interspecific differences in activity when animals were "alone" were, however, significant. *S. caecutiens* was most active, *S. unguiculatus* was least active, and *S. gracillimus* was intermediate between them. Dominance relationships between two individuals (win, even, lose, or no match) also had no effect on activities of shrews.

2. Experiment 2

The use of "home" or "away" cage did not differ significantly among species when shrews were "alone" (ANOVA, α=0.05). *S. unguiculatus*, however, tended to stay in its "home" cage more than either of the other two species (Table 2). The experimental treatments (alone and with con- or hetero-specific individuals) also had no effect on the use of "home" and "away" cages for any of the three species (Table 2). The dominance relationships tended not to influence the use of either the "home" or "away" cage in the three species ; there were no significant differences for almost all comparisons.

When two individuals were introduced into two interconnected cages, they tended to stay in separate cages (Table 3). The mean percentage of time spent in a single cage or separate cages did not differ significantly among the experimental treatments.

Table 2. The utilization of "home" and "away" cages by three shrew species observed in Experiment 2 (mean percent frequency±SD). The mean percentages differed significantly neither between the experimental treatments within species nor between species when shrews were "alone" ($\alpha=0.05$, ANOVA, arcsine transformation, Scheffe's method).

Experimental treatment	Alone	With		
		S. u.	*S. c.*	*S. g.*
S. unguiculatus				
Home	70.7±28.3	76.9±20.5	64.2±36.5	72.1±29.1
Away	29.3	23.1	35.8	27.9
(*n*)	(10)	(10)	(10)	(10)
S. caecutiens				
Home	50.7±31.9	52.0±26.6	36.3±11.6	49.6±23.2
Away	49.3	48.0	63.7	50.4
(*n*)	(4)	(4)	(4)	(4)
S. gracillimus				
Home	56.5±29.7	68.0±7.9	48.2±29.4	48.2±18.5
Away	43.5	32.0	51.8	51.8
(*n*)	(4)	(4)	(5)	(4)

S. unguiculatus remained underground or dug soil significantly more fre quently ($\alpha=0.05$) than did either *S. caecutiens* or *S. gracillimus* when they were in their "home" cages (Fig. 4). The mean frequencies of the three behavioral categories, however, did not differ significantly among the three species when they were in "away" cages (Fig. 4).

S. unguiculatus was "active underground" significantly more frequently in its "home" cage than it was in the "away" cage under each of the experimental

Table 3. Occupation of cages by two shrews in Experiment 2 (mean percent frequency±SD of staying in the same cage and separate cages). The means did not differ significantly between any comparisons ($\alpha=0.05$, ANOVA, arcsine transformation, Scheffe's method). *n*: number of experimental sessions examined.

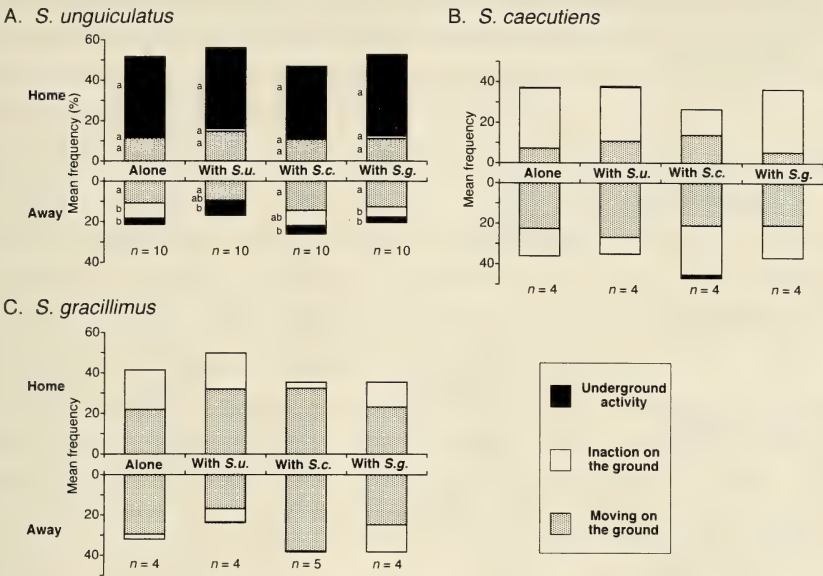|	With		
	S. unguiculatus	*S. caecutiens*	*S. gracillimus*
S. unguiculatus			
Same	35.9±19.9	35.6±17.3	24.2±14.3
Different	64.1	64.4	75.8
(*n*)	(5)	(10)	(10)
S. caecutiens			
Same	–	33.9±19.6	38.4±15.0
Different	–	66.1	66.3
(*n*)		(5)	(9)
S. gracillimus			
Same	–	–	54.8±10.7
Different	–	–	45.2
(*n*)			(4)

Fig. 4. The effects of con-or hetero-specific individuals on the behavior of shrews (mean percent frequency). The same letters indicate non-significant difference in mean frequency of each behavior category among the experimental treatments and between "home" and "away" cages within experimental treatment ($\alpha = 0.05$, ANOVA, Scheffe's method). In *S. caecutiens* and *S. gracillimus*, any significant difference in behavioral category was not found among the experimental treatments nor between "home" and "away" cages.

treatments (Fig. 4-A). In contrast, the mean frequency of each behavior in *S. caecutiens* and *S. gracillimus* did not differ significantly between the "home" and "away" cage situations (Fig. 4-B, C). The experimental treatments (alone and with con- or hetero-specific individuals) also had no effect on the behavioral patterns for any of the three species (Fig. 4).

The relationship between the mean frequencies of the behavioral categories and the dominance relation was not fully analyzed because of small sample size. However, behavioral patterns appeared not to be affected by the dominance relationship.

3. Dominance relationships and attacks

Among the three species, *S. unguiculatus* was most dominant and *S. gracillimus* was most submissive in terms of physical superiority. *S. unguiculatus* was seldom defeated by *S. caecutiens* and never defeated by *S. gracillimus* (Table 4). Furthermore, "no match" was the major result between conspecific individuals of *S. unguiculatus* in Experiment 2, but this result might be an artifact of the observation method that underground behaviors could not be observed. *S. caecutiens* beat *S. gracillimus* in most combats.

S. unguiculatus showed no significant difference in the number of attacks

Table 4.   Dominance relations between two con- or hetero-specific individuals in Experiments 1 and 2 (numbers of individuals of four kinds of the relation). Results of different experimental sessions for an individual were treated as diffrent counts.

		Opponent		
		S. u.	*S. c.*	*S. g.*
Experiment 1				
S. unguiculatus	win	4	6	6
	even	4	4	1
	lose	4	0	0
	no match	0	2	3
S. caecutiens	win	0	5	4
	even	4	4	2
	lose	6	5	1
	no match	2	0	2
S. gracillimus	win	0	1	2
	even	1	2	4
	lose	6	4	2
	no match	3	2	0
Experiment 2				
S. unguiculatus	win	1	6	8
	even	0	1	0
	lose	1	1	0
	no match	8	2	2
S. caecutiens	win	1	5	7
	even	1	0	0
	lose	6	5	0
	no match	2	0	2
S. gracillimus	win	0	0	4
	even	0	0	0
	lose	8	7	4
	no match	2	2	0

Table 5.   Mean numbers of attacks ($\pm SD$) between two individuals in Experiment 2.   The same letters indicate non-significant difference ($\alpha =$ 0.05, Mann-Whitney's U-test, the sequential Bonnferroni correction, Rice 1989).   The first letters before comma indicate the results of between-columm comparisons and the second letters are those of between-rows. Numbers parentheses are those of observations examined.

	Against		
	S. u.	*S. c.*	*S. g.*
S. unguiculatus			
	0.5±0.7 (2) a, a	1.2±1.6 (8) a, a	5.6±9.3 (8) a, a
S. caecutiens			
	1.0±1.4 (8) a, a	6.4±8.9 (10) a, a	27.4±16.6 (7) b, b
S. gracillimus			
	0.0±0.0 (8) a, a	0.6±0.5 (7) b, a	0.9±1.4 (8) ab, a

against other individuals (Table 5). *S. caecutiens* attacked *S. gracillimus* significantly more frequently than it did *S. unguiculatus* or other *S. caecutiens*. *S. gracillimus* attacked other individuals less frequently than did either of the other two species. *S. gracillimus* was attacked more often by *S. caecutiens* than by *S. unguiculatus* or by conspecifics (Table 5)

DISCUSSION

S. unguiculatus was frequently active underground, whereas *S. caecutiens* mainly used the ground surface (Figs. 3 and 4). *S. gracillimus* showed an intermediate vertical use of space in Experiment 1, but it was primarily a ground-surface dweller (Fig. 4) in Experiment 2, which was deemed to simulate natural conditions more realistically than Experiment 1.

The interspecific differences in use of space were consistent with those in burrowing habits (Ohdachi 1995c) and in dietary constituents (Abe 1968, Inoue and Maekawa 1990, Ohdachi 1995b) : *S. caecutiens* and *S. gracillimus*, which are poor burrowers and mainly eat small epigeal arthropods, showed more surface activity than did *S. unguiculatus*, which was a superior burrower and a heavy consumer of earthworms.

The presence of a con- or hetero-specific individual or their dominance relationships affected neither the space utilization nor the mean frequencies of behaviors (active/inactive and underground/resting/ground surface activity) in each of the three shrew species. *S. unguiculatus* was intrinsically different in its use of space (especially vertically) from *S. caecutiens* and *S. gracillimus*. It is, therefore, likely that direct interaction or interference over space is less frequent between *S. unguiculatus* and either of *S. caecutiens* or *S. gracillimus* than between the latter two species.

A dominance order among the three species was evident (Table 4) and seemed to correspond with the shrews' body size. The strongest *S. unguiculatus* weighs on average approximately twice as much as the second-ranked *S. caecutiens*, and *S. caecutiens* is 1.5 times as heavy as the weakest *S. gracillimus* (Ohdachi and Maekawa 1990b). The correlation between fighting ability and body size has also been reported from some other insectivorous or carnivorous vertebrates (*e.g.*, Persson 1985, Alatalo and Moreno 1987, Dickman 1988, Erlinge and Sandell 1988, Ducey *et al*. 1994, Nakano and Furukawa-Tanaka 1994).

Each of the three shrew species exhibited antagonistic behavior whenever two con- or hetero-specific individuals encountered, although *S. gracillimus* were least likely to attack. It may have been this tendency that led them to remain in whichever cage was not occupied by its opponent (Table 3). Many other soricine species also show antagonism against con- or hetero-specific individuals (Crowcroft 1957, Olsen 1969, Hawes 1977, Martin 1981, Barnard and Brown 1982, Churchfield 1990, Ellenbroek 1990, Dickman 1991, Ellenbroek and Hamburger 1991, Krushinska and Rychlik 1993). However, some species, such as *Neomys anomalus* and *Cryptotis parva*, are tolerant towards conspecifics

(Broadbooks 1952, Conaway 1958, Mock 1982, Krushinska and Pucek 1989, Krushinska and Rychlik 1993).   Krushinska and Pucek (1989) reported that acquaintance reactions, such as warning and nasal contact, were observed in *N. anomalus* when two individuals met.   In their study, shrews gradually avoided direct conflict by learning their place of the dominance rank.   In the present study, such acquaintance behaviors were not observed; shrews suddenly attacked other individuals (or were attacked) throughout experiments.   The lack of acquaintance behavior in the present study might have resulted from the brevity of experiments which might have led to their intolerance of other individuals.

Although *S. unguiculatus* was strongest of the three species (Table 4), it attacked other two species less frequently (Table 5).   In the present study, attacks could only be observed among animals on the ground surface, which might thus underestimate the attacking frequency of *S. unguiculatus*.   Under natural conditions, however, attacks by *S. unguiculatus* against *S. caecutiens* and *S. gracillimus* are also probably rare, because the latter two species use subterranean space less frequently and presumably rarely encounter *S. unguiculatus*.

Soricids usually establish intraspecific territories or exclusive home ranges, especially among individuals of the same sex (Ingles 1961, Shillito 1963, Buckner 1966, 1969, Croin-Michielsen 1966, Platt 1976, Hawes 1977, Pernetta 1977, Inoue 1988, 1991, Ohdachi 1992, Ivanter *et al*. 1994, Moraleva and Telitzina 1994, Stockley *et al*. 1994).   Such territoriality seems to be maintained by aggressive behavior and odor marking (Crowcroft 1957, Hawes 1976).   Two types of interspecific spatial relationships are known among soricine shrews. In the first type, territories overlap between species, as between *S. araneus* and *S. minutus* (Croin-Michielsen 1966, Pernetta 1977, Ellenbroek 1980).   In the second type, there is interspecific territoriality as between *S. cinereus* and *S. vagrans* (Spencer and Pettus 1966) and between *S. vagrans* and *S. obscurus* (Hawes 1977).   In Hokkaido, *S. unguiculatus* and either of *S. gracillimus* or *S. caecutiens* appear to have overlapped territories (Ohdachi 1992).   The occurrence of overlapped territories might be explained by the interspecific difference in vertical space use : *S. unguiculatus* appears only rarely to encounter either *S. gracillimus* or *S. caecutiens* in the field.   In contrast, inferring from the results of the present study (Figs. 3 and 4), it is plausible that *S. caecutiens* and *S. gracillimus* maintain interspecific territories when in symtopy, because both species are ground-surface dwellers and they do not shift their space of activity even when they co-habitate.

S. caecutiens tenaciously attacks *S. gracillimus*, and the latter seldom beats *S. caecutiens*.   The similarity in space use and the physical inferiority of *S. gracillimus* could lead to its exclusion from habitats where *S. caecutiens* occurs. Moreover, recipients of aggressive behavior may experience reduced fitness in general (King 1973).   This could partly explain the relative abundances of the two species in a given habitat (Ohdachi and Maekawa 1990a) : *S. caecutiens* and *S. gracillimus* do not occur together as the first and second most abundant

species. However, if *S. caecutiens* were to always exclude *S. gracillimus*, then *S. gracillimus* would be unable to occur in Hokkaido. In reality, *S. gracillimus* outnumbers *S. caecutiens* and *S. unguiculatus* in some habitats (Ohdachi and Maekawa 1990a). This might be attributed to interspecific differences in habitat preference. *S. gracillimus* is the most abundant species in moor and uplands, especially, in northern Hokkaido, whereas *S. caecutiens* tends to outnumber other species in habitats with sandy- or volcanic ash-soils (Ohdachi and Maekawa 1990a), which implies that each species prefers particular environments. Furthermore, competitive (interference) capabilities may vary in relation to such environmental variables as temperature, humidity, or soil type, and the result of competition depends on environmental conditions. Such phenomena are known in fish (Dunson and Travis 1991, De Staso and Rahel 1994), planktons (Hessen *et al.* 1995), and beetles (Park 1954). Also, the distribution pattern of soricids in Hokkaido is probably determined by a combination of both competitive ability and environmental conditions. In order to understand community organization or distribution pattern of the shrews in Hokkaido, further investigations of the effect of environmental conditions on competitive ability are recommended.

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The impact of forestry on the small rodent community of Hokkaido, Japan

Takashi SAITOH[1] and Atsushi NAKATSU[2]

Hokkaido Research Centre, Forestry and Forest Products Research Institute, Hitsujigaoka 7, Toyohira, Sapporo 062, Japan
Fax. +81-11-851-4167, [1]e-mail. bedford@ffpri-hkd. affrc. go. jp
[2]e-mail. nakatsua@ffpri-hkd. affrc. go. jp

Abstract.   The structure of small rodent communities, in both natural forests and young plantations, in the Asahikawa region of Hokkaido, Japan, in relation to the effects of long-term and large-scaled forestry, was analyzed using census data spanning the 31 years from 1962 to 1992.   Rodent communities in both natural forests and plantations consisted largely of four species : *Clethrionomys rufocanus*, *C. rutilus*, *Apodemus argenteus*, and *A. speciosus*.   *Clethrionomys rufocanus* was found to be dominant in both habitats, however the relative abundance of species differed significantly between habitats.   Although the dominancy of *C. rufocanus* was most obvious in forestry plantations, the proportion it contributed to the community decreased during the 1980s.   Conversely, *Apodemus* species have increased in both habitats over the same period.   Rodent species diversity has increased in the last decade.   The decline in the proportion of *C. rufocanus* has occurred in parallel with the decrease in the area of land under forestry plantation, which is the preferred habitat for *C. rufocanus*.   These findings indicate that monocultural habitats, such as forestry plantations, may support super dominant species such as *C. rufocanus*, which results in an impoverished rodent community, in terms of species diversity.

Key words : *Apodemus*, *Clethrionomys*, forest structure, species diversity.

Intensive silvicultural practices including site preparation, removal of potentially competing species, replacement of naturally occurring diversity with single species, and extensive use of herbicides, fertilizer sand pesticides, transform natural ecosystems into a timber production system.   Monotonous forestry plantation is, in other words, an artificially transformed, and greatly simplified habitat for animals.   The faunas of such artificial forests have been intensively investigated for comparison with those of natural forests, and the effects of the introduction of monoculture on faunas have been widely discussed.   Most previous studies have, however, been of a short-term nature, and on a limited spatial scale.

  Bird densities and species diversity are, for example, generally lower in plantations, especially in young plantations, than in natural forests (Fujimaki

1970, Ishigaki and Matsuoka 1972, Ishigaki *et al.* 1973, Kobayashi and Fujimaki 1985, Yui and Suzuki 1987 ; see also Murai and Higuchi 1988 for a review). Small mammal faunas also differ between natural and planted forests. Even when the species composition remains similar, the dominancy of the predominant species, for example, is enhanced in forestry plantations (Ota *et al.* 1977, see also Ota 1984 for a review). Increases in the abundance of specific vertebrate species have also been observed on areas of forest clear-cuts (Hansson 1994). Thus, a forest managed for maximum timber yield may be best regarded as analogous, in ecosystem terms, to a monocultured wheat field (Meffe and Carrol 1994). If the scale of operation was extended, so that the landscape became thoroughly transformed over several decades, then the effects of the plantation would be likely to become profound. Little is known, however, about such effects, because study on them requires wide-ranging long-termed investigation.

In the 1950s, the Japanese Forestry Agency investigated a policy to transform natural forests into more productive plantations. As a consequence of this policy, considerable areas of natural forests were clear-cut and transformed into single species, largely coniferous, plantations. In Hokkaido, Japan's northernmost island, this policy was implemented faithfully and extensively. During the peak period, more than one percent of natural forests were cut and transformed into coniferous plantations each year.

Because young plantations were frequently damaged by the grey-sided vole, *Clethrionomys rufocanus* Sundevall, the Forestry Agency has, since 1954, carried out censuses of small rodents, for management purposes, in forests all over Hokkaido. That census data have proven invaluable. It has enabled us to describe, in this paper, changes in the structure of small rodent communities, in both natural forests and young plantations in Hokkaido over three decades from 1962 to 1992, and to analyze the effects of long-term, large-scaled plantations on small rodent communities.

MATERIALS AND METHODS

1. Study area and census methods

Since 1954 the Forestry Agency has carried out censuses of rodent populations at approximately 1,000 sampling locations all over the 78,073 km² island of Hokkaido (41°24′–45°31′N, 139°50′–145°49′E). The forests under Forestry Agency Management cover 28,300 km² (21,500 km² of natural forests, and 6,800 km² of plantations). In 1990, these forests were managed by 81 District Offices, which were subdivided into Ranger Offices. The censuses were carried out by each individual Ranger Office. The data analyzed for this paper come from 8,034 km² of northern Hokkaido (corresponding to about 10% of the island's total area) under the supervision of the Asahikawa Sub-regional Office. Our study areas included 22 District Offices, which consisted of 113 Ranger Offices in 1990. Most of the natural forests in this area are classified as "pan mixed forests" with needle and broad-leaved trees, in what is regarded as a transition

zone between the temperate and boreal zones (Tatewaki 1958). The dominant tree genera here are: *Abies*, *Acer*, *Betula*, *Picea* and *Quercus* (Tatewaki 1958).

Trapping was carried out three times a year, in spring (May or June), summer (July or August) and autumn (September or October). Ranger Offices set 50 snap traps, at 10 m intervals on 0.5 ha (50×100 m) grids, over either three or five consecutive nights. Since rodent abundance in Hokkaido usually reaches its peak in autumn, and because autumn populations are known to reflect accurately annual variation in populations (Saitoh 1987), we used autumn census data in our analyses.

Four rodent species, *C. rufocanus*, *C. rutilus* (Pallas), *Apodemus speciosus* (Temminck), and *A. argenteus* (Temminck) were recorded during the censuses. *C. rex Imaizumi*, *A. peninsulae* (Thomas), and several species of shrew, *Sorex* spp., also occur in the region and may have been caught occasionally. These less common species were, however, not reported officially.

Each Ranger Office censused 2–6 separate sites. Census grids were usually located in selected habitats (young plantations, and natural forests neighbouring plantations) which together constituted a unit. Census sites were sometimes relocated within the area of a given Ranger Office, and methods have changed over the period of study. From 1962 to 1976, for example, traps were set for five nights, whereas from 1977 to 1992 a three-night trapping scheme was employed. To make these two data sets compatible for time-series analyses, the data from the first 15 years were transformed to three-night equivalents (*i.e.*, 150 trap nights), using the regression of the three-night (y) on the five-night (x) captures ($y = 0.68066x + 0.18127$, $r^2 = 0.935$).

For the purposes of this study, rodent abundance is defined as the number of individuals caught per 150-trap-nights. Species diversity and species ratios were calculated based on the data from the four main rodent species. The Shannon-Wiener function (index of evenness, J') was used as an index for species diversity (Krebs 1989). As data for 1970 and 1974 have been lost (except for on *C. rufocanus*), values have been calculated excluding the data from these two years. Moving averages, for each three year period, were used to smooth annual variation. For the calculation of moving averages, for periods including the years 1962, 1970, 1974, and 1992, values were obtained from the data for the associated two years.

2. Forest management

Forest planting follows several silvicultural procedures. For this paper we focused on the most drastic method of transforming a natural system into an artificial one; that is young plantations grown on clear-cuts. After clearance of natural forest, weeds are removed from the clear-cut, then larch, *Larix kaempferi* (Lambert) or fir, *Abies sachalinensis* Fr. Schmidt seedlings are planted densely. Because weeds grow thickly in young plantations and may suppress the growth of tree seedlings, the weeds are cut every summer until between five and nine years after tree planting. Censuses were carried out in such young plantations until ten years after planting.

3. The voles and mice

The grey-sided vole, *Chlethrionomys. rufocanus*, is common in both open fields and in natural forests and plantations in Hokkaido.   This species is well known for exhibiting a wide spectrum of population dynamics ranging from stable to cyclic (Saitoh 1987, Bjørnstad *et al*. 1996, Stenseth *et al*. 1996, Saitoh *et al*. 1997).   This small (30–40 g), short-tailed (around 40% of head and body) rodent is more folivorous than other *Clethrionomys* species (Hansson 1985). This feeding habit is particularly prevalent in Hokkaido, possibly due to the absence of *Microtus* spp.   This is consistent with its wider habitat preference from open fields to natural forests in Hokkaido.   During winter, *C. rufocanus* eats mainly leaves and shoots of bamboo grass, and some shrub/tree bark. During summer it eats various forbs and grasses, and in autumn, acorns are eaten to some extent (Ota 1984).

The red-backed vole, *C. rutilus*, is a short-tailed, forest-dwelling rodent. Its body shape is similar to that of *C. rufocanus*, though at 20–30 g, it is smaller. Although *C. rutilus* is essentially granivorous (Hansson 1985), it also eats, to some extent, insects year around (Ota 1984).   Its abundance is usually low in Hokkaido, though it sometimes dominates in mature coniferous forests (Ota 1984).

The Japanese wood mouse, *A. argenteus*, is endemic to Japan (though ecologically equivalent to *A. sylvaticus* of Eurasia).   It is small, weighing just 15–20 g, and has a relatively long tail which is longer than its body length.   At a weight of 40–60 g, *A. speciosus*, another Japanese endemic mouse, is the largest of the four species analyzed here.   Its tail is relatively short (77–99% of body length) for a mouse-shaped rodent.   These two *Apodemus* species are both largely granivorous, though they also eat a considerable amount of insects (Ota 1984).   The larger species *A. speciosus* prefers larger seeds such as acorns, walnuts, or pine nuts, whereas *A. argenteus* eats smaller seeds and berries.

The main habitats of these two species include various forest types.   The two species are usually dominant in natural forests, though *A. speciosus* is also found in open fields.

RESULTS

1. Species composition

The total of 223,663 rodents were trapped during the 31 year study period ; 122,653 of these were from 6,438 census grids in natural forests, and 101,010 were from 5,222 grids in young plantations.   The average number per trapping grid in the two types of forests were very similar : 19.1 for natural forests and 19.3 for plantations.

The proportion of *Clethrionomys rufocanus* to the total number of rodents captured, exceeded 50% in both natural forests and plantations (Fig. 1). Although the order of dominance (*C. rufocanus* > *A. argenteus* > *A. speciosus* > *C. rutilus*) was the same in natural forests and in plantations, relative proportions of each species differed significantly between them (*G*-test, $G_{adj} = 449.3$,
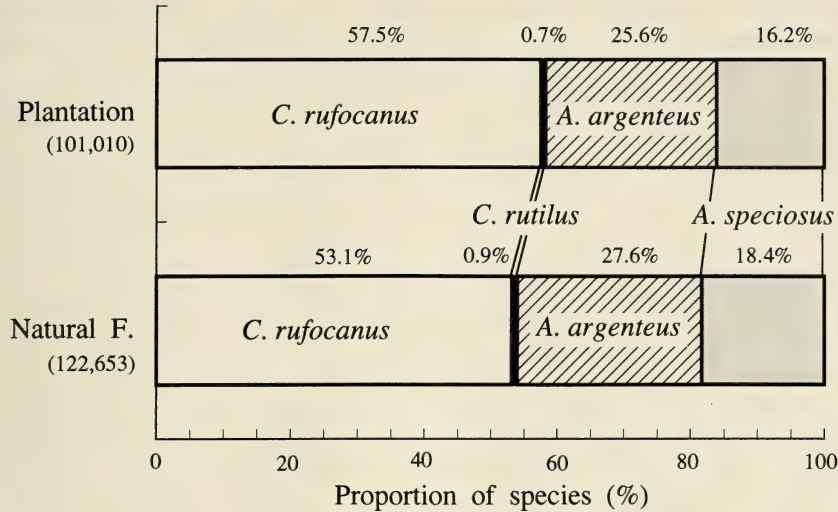
Fig. 1. The proportion of rodent species in natural forests and plantations in the Asahikawa region, Hokkaido, Japan. Figures in parentheses indicate the total number of rodents caught during the 31 year census.

$p < 0.001$, Sokal and Rolf 1995). The dominancy of *C. rufocanus* was particularly obvious in plantations. Because *C. rutilus* was very uncommon, our main analyses are of the other three species.

2. Variation in species abundance

Rodent abundance fluctuated greatly from year to year, particularly at two to four year intervals, both in natural forests and in plantations (Figs. 2a, 2b). This pattern may be led by the demographic features of the dominant species, *Clethrionomys rufocanus*. Basic statistics of population dynamics are given in Table 1. Note that values indicating variability (*i.e.*, CV, s-value, and Max/Min ratios) were moderated owing to averaging the abundance of rodents on more than 100 census grids.

The relative proportion of *C. rufocanus* was correlated with its abundance, whereas this relationship was not found in other species, with the exception of *A. speciosus* in plantations (Table 2). These vague relationships among *Apodemus* species, attributed to the positive correlation in abundance with *C. rufocanus*, which was most influential on the proportions of the various species (Table 3). Even when the abundance of an *Apodemus* species increased, it still did not represent a large proportion of the community because *C. rufocanus* was always even more abundant. The abundances of the three main species were generally correlated with each other in both natural forests and plantations (Table 3).

Table 1. Basic statistics for rodent abundance. Data on *C. rutilus* was eliminated because of its scarcity. Note that values indicating variability (*i.e.*, *CV*, Max/Min ratio and *s*-value) were moderated owing to averaged figures.

	C. rufocanus	*A. argenteus*	*A. speciosus*
Natural forests			
Average	9.49	5.16	3.55
CV (%)	51.90	41.30	62.20
Max.	19.28	10.21	10.40
Min.	1.49	1.25	0.67
Max./Min.	12.95	8.16	15.42
s-value	0.29	0.21	0.28
Plantations			
Average	10.69	4.87	3.11
CV (%)	53.30	44.10	62.40
Max.	22.52	10.92	9.80
Min.	1.31	1.14	0.63
Max./Min.	17.24	9.59	15.55
s-value	0.31	0.22	0.29

Table 2. Relationships between abundance and proportion in the three species are given using Kendall rank-order correlation coefficient τ ($n=29$). Figures in parentheses are probabilities of a Type I error for Kendall's τ.

	C. rufocanus	*A. argenteus*	*A. speciosus*
Natural forest	0.468	0.094	0.217
	(0.000)	(0.476)	(0.099)
Plantations	0.429	0.244	0.274
	(0.001)	(0.063)	(0.037)

Species proportions were, however, negatively correlated between *C. rufocanus* and the two *podemus* species, whereas a positive relatonship was found between the two *Apodemus* species (Table 3). Positive correlations between the two *Apodemus* species, both in abundance and species proportion, indicate that competition between them is probably not severe. Species proportions fluctuted from year to year with some clear patterns revealed by moving averages (Figs. 2a, 2b). *C. rufocanus* seemed to have gradually lost its dominancy in both natural forests and plantations since the 1980s (Fig. 3a, 3b). In contrant to the decline in *C. rufocanus, Apodemus* species contributed a steadily increasing proportion of the community in the later years of the study.

3. Species diversity

Species diversity values in natural forests fluctuated around 0.7 during the 1960s and early 1970s, increased fro the late 1970s to the early 1980s, and thereafter attained relative stability at 0.8. The change in species diversity in plantations exhibited a very similar pattern to that in natural forests, although
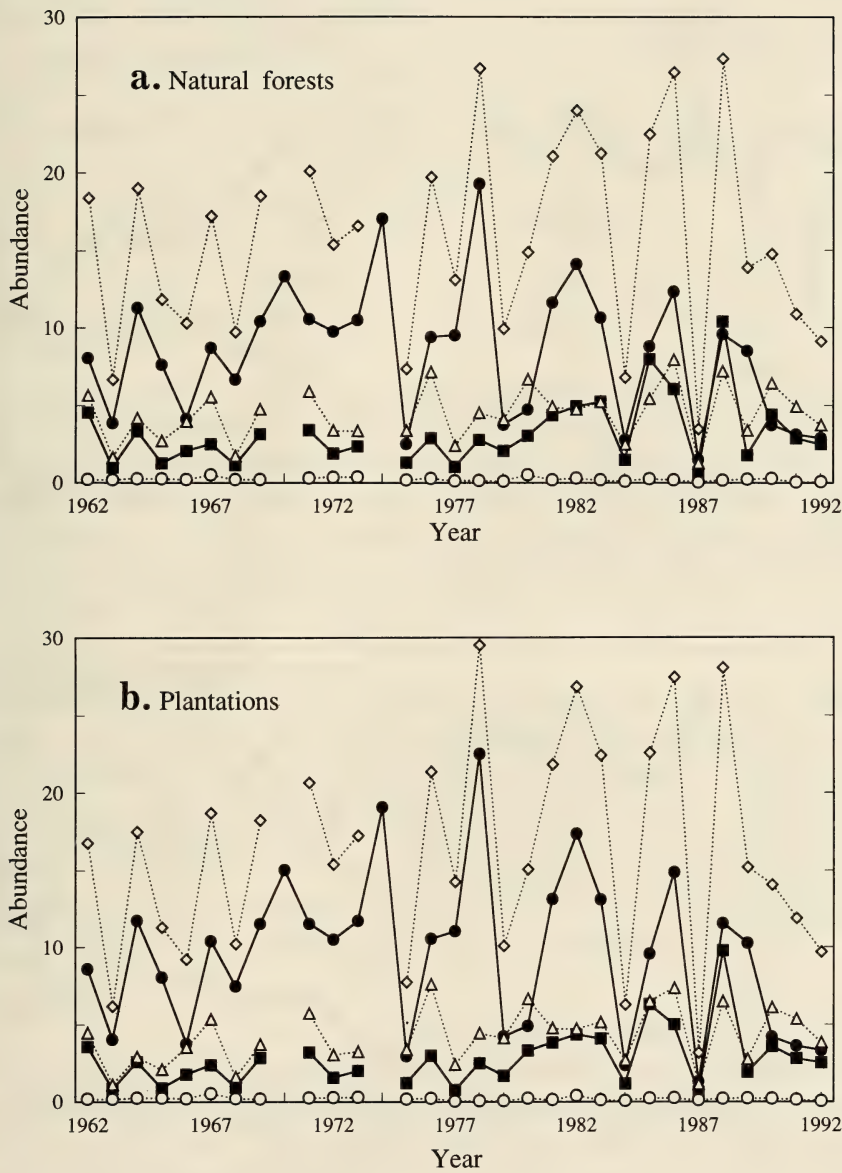
Fig. 2. Fluctuation of rodent abundance in: a. natural forests and b. new plantations. Abundance is shown as the number of rodents caught per 150-trap night. Lozenge: the total number, solid circle: *C. rufocanus*, triangle: *A. argenteus*, square: *A. speciosus*, and open circle: *C. rutilus*.
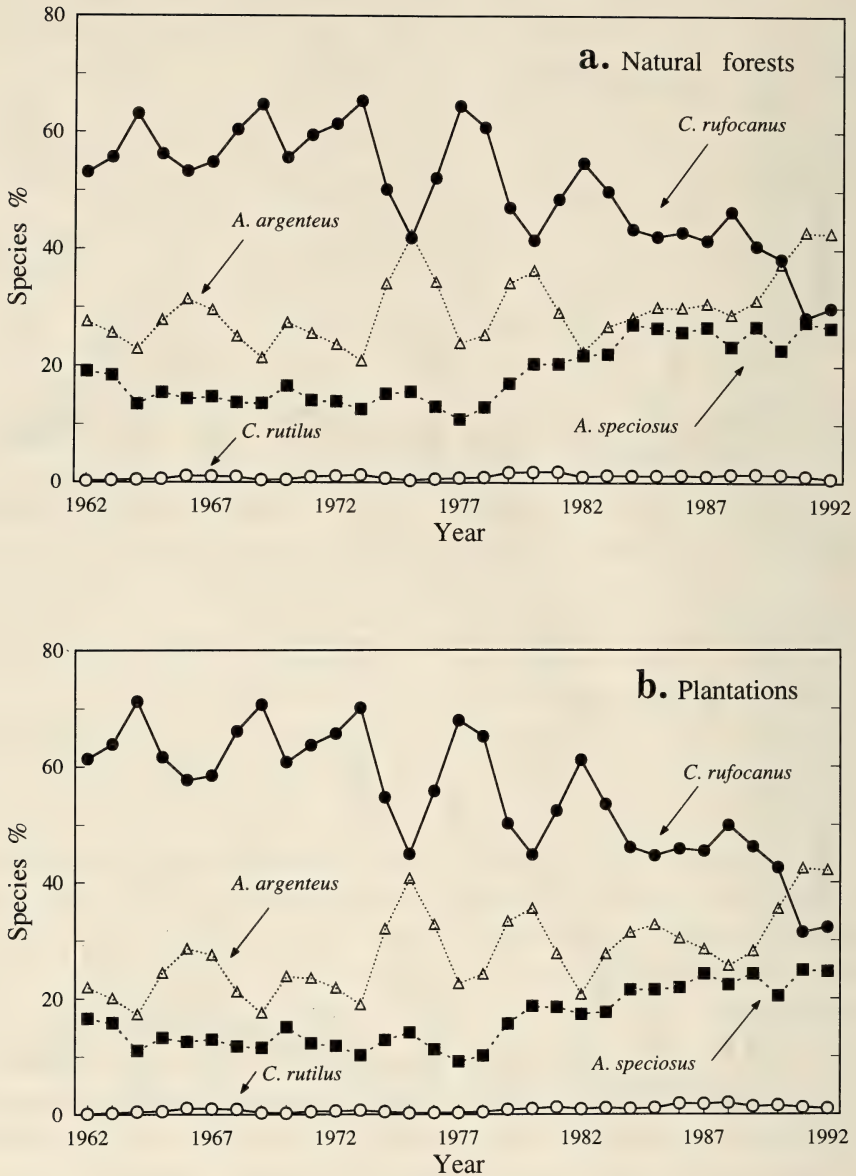
Fig. 3.   Changes in rodent species ratios in: a. natural forests, and b. new plantations. Species proportions are shown with moving averages for each three year period.   Solid circle: *C. rufocanus*, triangle: *A. argenteus*, square: *A. speciosus*, open circle: *C. rutilus*.

Table 3. Relationships of abundance and proportion between the three species of rodents (*C. rufocanus* [*Cr*], *A. argenteus* [*Aa*], and *A. speciosus* [*As*]) in the two types of forests are given using Kendall rank-order correlation coefficient τ ($n=29$). Figures in parentheses are probabilities of a Type I error for Kendall's τ. Upper matrix for natural forests, lower matrix for plantations.

	Abundance			Proportion		
	Cr	*Aa*	*As*	*Cr*	*Aa*	*As*
Cr	—	0.301 (0.022)	0.281 (0.032)	—	−0.709 (0.000)	−0.668 (0.000)
Aa	0.222 (0.091)	—	0.655 (0.000)	−0.778 (0.000)	—	0.387 (0.003)
As	0.266 (0.043)	0.640 (0.000)	—	−0.699 (0.000)	0.478 (0.000)	—

the species diversity in plantations was almost always lower than that in natural forests (Fig. 4, Wilcoxon signed-ranks test, $Z=-4.249$, $p<0.0001$). Species diversity in natural forests during the latest ten years averaged 0.8, which was significantly higher than during the first ten years (0.7, Random permutation test, $p=0.001$). A similar significant pattern was also observed in plantations, where species diversity averaged 0.6 in the first decade, and 0.8 in the latest (Random permutation test, $p=0.0012$).

4. Species diversity and forestry

Extensive forest planting took place during the 1960s and early 1970s in Hokkaido. More than one percent of natural forests (more than 7,000 ha) were felled, and coniferous seedlings were planted on the clear-cuts within a year. Since the 1970s, however, planting effort has decreased (Fig. 4). The pattern of planting has been closely followed by the proportion of the small rodent community contributed by *C. rufocanus* (Fig. 3). The proportion of *C. rufocanus* was highly correlated with the area of new plantations (Kendall's $\tau=$ 0.897, $p<0.001$ for natural forests; $\tau=0.566$, $p<0.001$ for plantations).

DISCUSSION

The gray-sided vole, *C. rufocanus*, was found to be the most abundant small rodent in both natural forests and plantations (Fig. 1). Its dominancy was most obvious in plantations. The young plantations, where the censuses were carried out, were open and herb-dense habitats and the preferred habitat of *C. rufocanus* in Hokkaido (Ota 1984). Thus, the dominancy of *C. rufocanus* in plantations is consistent with previous studies (Ota *et al*. 1977, Ota 1984). The present results, indicating that *C. rufocanus* contributed over 50% of small rodent communities even in natural forests, should, however, be noted. Previous studies have indicated that either *A. argenteus*, or *A. speciosus* is usually
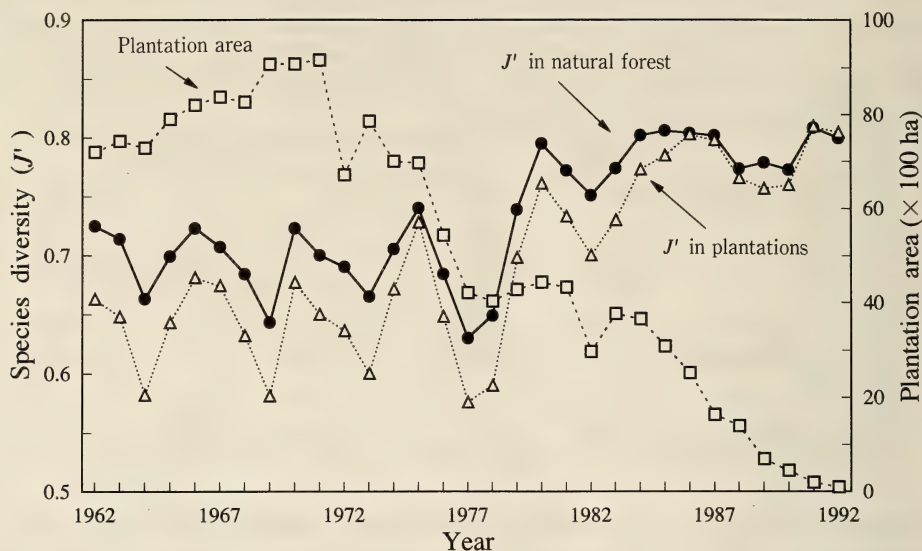
Fig. 4. Changes in rodent species diversity and in the area of new plantations. The Shannon-Wiener function (index of evenness, J') was used as an index for species diversity. Species diversity is shown with moving averages for each three year period. Circle : species diversity in natural forests, triangle : species diversity in plantations, square : new plantation area.

dominant in mature natural forests, even though *C. rufocanus* is also common there (Ota *et al.* 1977, Ota 1984). The extensive areas of plantation contiguous with the natural forests studied here may have led to the increased proportion of *C. rufocanus* in natural forests.

The prominent dominancy of *C. rufocanus* caused species diversity to be low during the 1960s and early 1970s (Figs. 3, 4). Thereafter, as the proportion of *C. rufocanus* decreased, species diversity increased. These changes were consistent with changes in forestry planting effort. These findings suggest that monocultural habitats, such as forestry plantations, may support a super dominant species (in this case *C. rufocanus*), which suppresses species diversity in the rodent community.

The censuses were carried out continuously on the same types of habitats (on young plantations and on natural forests neighbouring plantations) throughout the study period. Thus the decrease in the relative proportion of *C. rufocanus* was not caused by environmental changes on census grids. The present results should, therefore, reflect changes in rodent communities in more extensive areas than just on census grids ; thereby indicating that long-term, extensive forestry practices may simplify the rodent community not merely in the plantations themselves but also in the natural forests surrounding large scale plantations.

Nakatsu (1988, 1992) has also reported changes in species proportion based

on the same census data that we analyzed; he did not analyze the data as a time-series, but his data set, however, covered all regions of Hokkaido. Although Nakatsu (1988, 1992) also found a significant reduction in the proportion of *C. rufocanus* in the Asahikawa region, he did not find such a reduction in either Kitami or Obihiro regions, where planting efforts were also decreasing during the 1980s.

A clear relationship between rodent communities and forestry plantations was revealed in this study. We do not think that this relationship is superficial, and believe that extensive forestry planting may simplify the rodent community on a large scale. The present analyses are not robust enough, however, to prove this, because different types of rodent population fluctuations were pooled in this study (see Bjørnstad *et al.* 1996), and because this study tells nothing about the regional differences in species proportion that Nakatsu (1988, 1992) observed. To resolve these problems more detailed analyses are required.

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Short Communication

Growth of eye lens weight and age estimation in the northern red-backed vole, *Clethrionomys rutilus*

Kenichi TAKAHASHI and Kei SATOH

Hokkaido Institute of Public Health, N19 W12 Kita-ku, Sapporo 060, Japan
Fax. +81-11-736-9476, e-mail. takaken@iph. pref. hokkaido. jp

Age determination is a basic requirement when analyzing the ecological events affecting wild animals. Several useful methods for age determination have been reported for small rodents (see Pucek and Lowe 1975). Tooth wear patterns and the molar root ratio have often been used to assess the ages of Japanese rodents (Abe 1976, Fujimaki *et al*. 1976, Fujimaki 1977, Hikida and Murakami 1980). Furthermore, since Lord (1959) proposed a method of age determination using the eye lens weight (ELW) in cottontail rabbits, *Lepus americanus*, it has become well-known that ELW can also be employed as an age criterion in various species of Rodentia (Östbye and Semb-Johansson 1970, Gourley and Jannett 1975, Hagen *et al*. 1980, Thomas and Bellis 1980, Ando and Shiraishi 1997 for the subfamily Microtinae, and Berry and Truslove 1968, Adamczewska-Andrzejewska 1973, Yabe 1979, Okamoto 1980, Takada 1982, Hardy *et al*. 1983, Takada 1996 for the subfamily Murinae). It is considered that the ELW method of age estimation is more accurate than any other technique relying on body or skull measurements (Pucek and Lowe 1975). Moreover, this method has the advantage that it can be used for species which have rootless molars such as *Eothenomys smithii* (Ando and Shiraishi 1997).

The growth of the molar roots of the northern red-backed vole, *Clethrionomys rutilus* was examined as an indicator of absolute age by Tupikova *et al*. (1968), and the relationship between lens weight and age was analyzed using specimens captured in the field (Askaner and Hansson 1967), however, no previous data on the growth patterns of eye lens from known-age individuals have been reported for this species.

An accurate method for age determination is of value not only for ecological studies of *C. rutilus* itself, but also for analysis of the transmission pattern of a zoonosis in a natural population. The latter is of particular significance because *C. rutilus* is one of the favorable intermediate hosts of *Echinococcus multilocularis*, a parasitic organism causing the serious disease alveolar echinococcosis in humans, which has been found in Hokkaido, Japan (Takahashi and Nakata 1995).

The purpose of the study described here, therefore, was to establish an age estimation equation by analyzing the relationship between the growth in eye lens weight and age in a population of known-age laboratory-reared northern

red-backed voles.

MATERIALS AND METHODS

A total of 197 voles (91 males and 106 females) from a laboratory colony originating from wild voles captured in Sapporo, Hokkaido were used in this study.   The laboratory colony was maintained under regulated conditions at a temperature of 23-25°C a 12 hour light and 12 hour dark photoperiod and fed a commercial diet (CMF, Oriental Yeast Co. Ltd.).   Voles were reared individually in mouse cages except for during breeding.   Male voles ranged in age from 15 to 596 days, and females from 15 to 581 days.   Voles were killed with ethyl ether, their eyes were dissected out and fixed in 10% formalin at room temperature for at least four weeks, then the optic lenses were excised and washed with distilled water.   Lenses were dried in an oven at 80°C for 24 hours and immediately weighed to the nearest 0.01 mg on a microbalance (Mettler, AT201).

RESULTS AND DISCUSSION

Lens weight was found to increase rapidly up to about day 50 and then the growth rate decreased gradually in both males and females (Fig. 1), as has also been noted for *Lemmus lemmus* (Östbye and Semb-Johansson 1970).   In this study, ages were selected non-randomly and measured without error.   For this reason, in the regression analysis of this data, age is the independent variable and lens weight the dependent variable with lens weight regressed on age (Hagen *et al.* 1980).   Ages were logarithmically transformed, because the growth pattern of lens weight was found to be curvilinear in *C. rutilus* (Fig. 1).
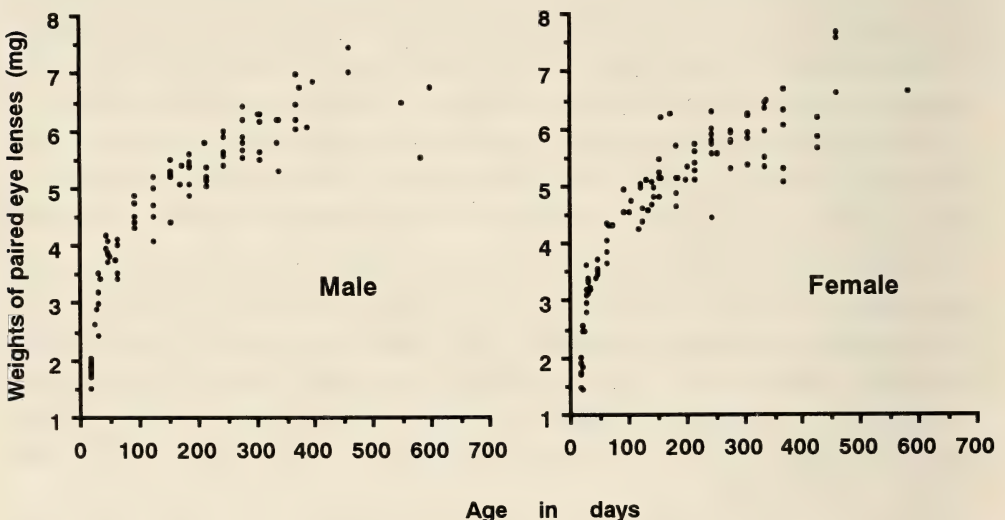

Fig. 1   Growth of the eye lens weight in 91 male and 106 female northern red-backed voles, *Clethrionomys rutilus*.
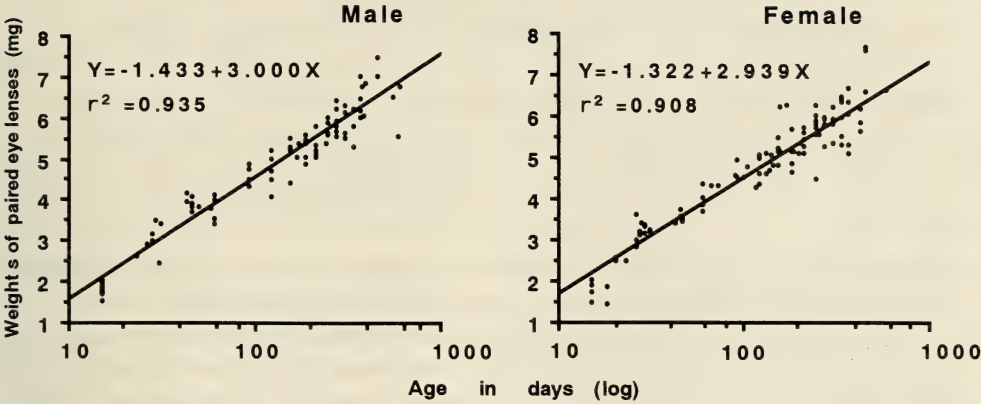
Fig. 2 Relationship between log-transformed ages and eye lens weight in the northern red-backed vole, *Clethrionomys rutilus*.

Moreover, array variance must be of equal magnitude along the length of the line (homoscedasticity) in the regression analysis (Dapson 1980), and homoscedasticity was confirmed with the graphs showing residuals plotted against Y_i (the Y value on the line X_i) for both sexes. The simple linear regression relationship ($Y = a + bX$) between X (age in days after logarithmic transformation) and Y (lens weight) was applied (Fig. 2). Regression equations from our data from *C. rutilus* were:

(1) $Y = -1.433 + 3.000X$ ($r^2 = 0.935$, $p < 0.001$) for males
(2) $Y = -1.322 + 2.939X$ ($r^2 = 0.908$, $p < 0.001$) for females

where $Y =$ weight of paired lenses in mg, $X = \log_{10}x$, and $x =$ age in days.
      There were no significant differences in regression slopes between males and females (F-test, $F_{cal} = 0.24$, $F_{0.05(1,193)} = 3.89$, $p > 0.05$) (Table 1). Age was predicted inversely from either equation (1) or (2), and predicted age was given by the equation:

$\hat{x} = 10^{(Y+1.433)/3.000}$ for males
$\hat{x} = 10^{(Y+1.322)/2.939}$ for females

      The equation of the 95% confidence limits (L) for the inverse prediction is given as follows:

Table 1. Statistics on the regression lines between the age and eye lens weight in the northern red-backed vole, *Clethrionomys rutilus*.

Sex	n	a	b	r	$\bar{X}$	$\bar{Y}$	S_{YX}	SS_X	t
Male	91	−1.433	3.000	0.967	2.064	4.760	0.382	20.638	1.987
Female	106	−1.322	2.939	0.953	2.061	4.736	0.426	21.630	1.983

n: number of samples, a: Y intercept, b: slope, r: correlation coefficient, $\bar{X}$: mean of X, Y: mean of Y, S_{YX}: standard error of estimate, SS_X: sum of squared deviations of X, t: Student's t ($d.f. = n-2$, $p = 0.05$).

$$L=\bar{X}+\frac{b(Y_i-\bar{Y})}{K}\pm\frac{t}{K}\sqrt{S^2_{YX}\left[\frac{(Y_i-\bar{Y})^2}{SS_X}+K\left(\frac{1}{m}+\frac{1}{n}\right)\right]}$$

where $\bar{X}=$the mean of X, $\bar{Y}=$the mean of Y, $K=b^2-t^2S_b^2$, $t=$Student's t ($d.f.=n-2$, $p=0.05$), $S_b=$the standard error of the regression coefficient, $S^2_{YX}=$ the residual mean square, $SS_X=$the sum of squared deviations of X, $n=$sample size, and $m=$the number of individuals upon which predictions will be based. Here, when $m=\infty$, L indicates the confidence limits of the mean prediction for the population. On the other hand, when $m=1$, L indicates the confidence limits of the individual prediction (Dapson 1980, Sokal and Rohlf 1981).

When estimating the ages of individual animals, Dapson (1980) pointed out the importance of presenting the 95% confidence limits, as the confidence interval indicates the accuracy of an estimate of age for each specimen, and the confidence interval for the individual prediction is generally broader than that for the population. This certainly proved to be the case in *C. rutilus* (Tables 2 and 3). In this study, broader ranges in the 95% confidence interval were observed among older animals because of the wide variance of lens weight and the decrease in the growth rate in these older animals (Fig. 1).

Askaner and Hansson (1967) examined the relation between ELW and molar root length of wild-caught *C. rutilus*, and pointed out the usefulness of the ELW method for aging individuals of this species. The present study provides, for the first time, an equation for age estimation based on ELWs of known-age voles. Tupikova *et al.* (1968) developed an age determination

Table 2. Predicted ages and confidence limits (95 %) for the mean and individual predictions at given lens weights in the male northern red-backed vole, *Clethrionomys rutilus*.

Lens weight (mg)	Predicted age in days	Mean predictions		Individual predictions	
		Lower age limit	Upper age limit	Lower age limit	Upper age limit
1.5	9	8	11	5	17
2.5	20	18	23	11	37
3.5	44	40	48	24	79
4.5	95	89	101	53	171
5.5	205	191	219	114	369
6.5	442	402	487	245	799

Table 3. Predicted ages and confidence limits (95 %) for the mean and individual predictions at given lens weights in the female northern red-backed vole, *Clethrionomys rutilus*.

Lens weight (mg)	Predicted age in days	Mean predictions		Individual predictions	
		Lower age limit	Upper age limit	Lower age limit	Upper age limit
1.5	9	7	10	4	18
2.5	19	17	22	10	38
3.5	43	39	47	22	83
4.5	94	88	100	48	182
5.5	205	191	221	105	400
6.5	451	406	502	230	883

method for *C. rutilus* using the length of the root and the height of the crown of M², however, since the neck of M² in this species is not formed until three months old, the ages of young voles under two months old cannot be predicted by this method. The present results show that the ELW technique is capable of estimating age in this species, especially in younger voles. For application of this technique to field studies, however, we must pay attention to the wide confidence interval in older voles.

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Age determination in the Smith's red-backed vole, *Eothenomys smithii*, using optic lens weight

Akiro ANDO[1] and Satoshi SHIRAISHI[2]

[1]*Department of Food Science, Shimane Prefectural Shimane Women's College, Matsue 690, Japan*
Fax. +81-852-21-8150
[2]*Zoological Laboratory, Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812-81, Japan*

Abstract.   A technique for age determination based on the dry weight of the optic lens was tested in Smith's red-backed vole, *Eothenomys smithii*.   The model equation $W = a + b\log_{10}A$ (W : lens weight in mg, A : age in days, a and b : parameters to be estimated from data) was applied to our data from 65 known-age laboratory-reared voles.   As a result, the predicted age in days ($\hat{A}$) at a given lens weight (W) could be calculated from the equation $\hat{A} = 10^{(W + 1.415)/2.131}$.   For example, an individual with a lens weighing 3.22 mg was estimated as having a predicted age of 150 days, and the 95% confidence interval at 150 days was calculated to be 17 days (142–159 days, or 11.3% of the predicted age) for the mean prediction and 144 days (94–238 days, or 96.0% of the predicted age) for the individual prediction.   Lens weight can, it appears, provide the best age criterion at present, particularly in rodents with rootless molars such as *E. smithii*.

Key words : age determination, *Eothenomys smithii*, lens weight, Microtinae, red-backed vole.

Information concerning age is a very important aspect of ecological studies of wild animals.   Many methods for age estimation have been proposed and used in various mammalian species (see Morris 1972 for review).   Among the various methods available, the lens weight technique has been evaluated as a useful and powerful technique in small to medium-sized mammals, such as rodents (Hagen *et al.* 1980 for *Microtus oeconomus*, Okamoto 1980 for *Rattus norvegicus*, Tanikawa 1993 for *Rattus rattus*, Takada 1982a for *Mus musculus molossinus*, Takada 1982b for *Apodemus speciosus*), and lagomorphs (Load 1959 for *Sylvilagus floridanus*, Dudzinski and Mykytowycz 1961 for *Oryctolagus cuniculus*, Connolly *et al.* 1969 for *Lepus californicus*, Bothma *et al.* 1972 for *S. floridanus*, Hearn and Mercer 1988 for *Lepus arcticus*, Ando *et al.* 1992 for *Lepus brachyurus*).

Smith's red-backed vole, *Eothenomys smithii*, of the subfamily Microtinae, is endemic to Japan, where it occurs widely in forested areas of Kyushu, Shikoku, and western and central Honshu (Kaneko 1992).   In contrast to the Japanese gray red-backed vole, *Clethrionomys rufocanus bedfordiae* (Abe 1976) and the large Japanese field mouse *A. speciosus* (Hikida and Murakami 1980),

neither the molar root ratio, nor the molar wear pattern, can be employed as an age criterion in *E. smithii*, since its molars are rootless and grow persistently. No information has been available on age estimation in *E. smithii*. For the experiment described here, age was estimated for individual *E. smithii*, based on the optic lens weight of known-age individuals, using statistical treatments recommended by Dapson (1980).

MATERIALS AND METHODS

1. Lenses

The *Eothenomys smithii* used for this study were obtained from a laboratory colony which originated from wild voles live-trapped on Mt. Wakasugi in Fukuoka Prefecture, northern Kyushu. The colony was maintained under controlled conditions, *i.e.*, temperatures of 15-20 °C and photoperiods of 12-13 hr light : 12-11 hr dark (Ando *et al.* 1988). A total of 65 voles (33 males, 32 females) ranging in age from 20 to 600 days were killed with ethyl ether. Both right and left eyes were dissected out and placed individually in 10% formalin for two to three weeks, then the optic lenses were carefully removed. After being rinsed with distilled water, the lenses were dried at 80 °C for two days and weighed to the nearest 0.01 mg on an analytic balance (Mettler AE-100). The combined dry weight of both right and left lenses of each individual vole was used for statistical analysis (Table 1).

Table 1. The combined lens weight of the right and left eyes in 65 known-age Smith's red-backed voles, *Eothenomys smithii*.

Age (days)	Lens weight (mg)	Age (days)	Lens weight (mg)	Age (days)	Lens weight (mg)
20	1.29	140	3.16	255	3.70
20	1.37	140	3.38	255	3.80
20	1.37	142	2.98	255	3.60
22	1.32	160	3.28	258	3.94
32	1.92	160	3.08	282	3.80
32	1.84	160	3.28	282	3.90
38	1.83	160	2.85	282	3.80
43	2.25	160	3.14	300	3.81
44	2.37	176	3.48	300	4.18
44	2.30	180	2.92	300	4.03
44	2.37	180	3.80	350	4.22
50	2.24	180	3.62	350	4.25
50	2.06	180	3.43	400	4.00
60	2.13	200	3.44	468	4.71
70	2.59	200	3.26	500	4.60
80	2.50	200	3.00	550	4.00
100	2.61	200	3.29	550	4.37
105	3.24	200	3.36	600	4.40
120	2.69	200	3.74	600	4.72
120	2.92	214	3.50	600	4.44
140	3.02	236	3.43	600	4.41
140	3.36	250	3.78		

2. Statistical procedure

The mathematical model for the relationship between lens weight (W) and age in days (A) pioneered by Hagen *et al.* (1980) and Takada (1982a, 1982b) was used for this study, *i.e.*,

$$W = a + b \log_{10} A \quad (1)$$

where a and b are parameters to be estimated from the data set. Here, when we define $W = Y$ and $\log_{10} A = X$ in this model, the equation (1) could be $Y = a + bX$, thereby, making linear regression analysis available for the relationship between Y (lens weight) and X (age in days after logarithmic transformation). In the regression analysis, data of X and Y should exhibit homoscedasticity (Dapson 1980, Zar 1984), which we confirmed for our data in accordance with Zar's (1984) procedure.

The linear regression equation $Y = a + bX$ refers to the regression of Y (the dependent variable) on X (the independent variable). When estimating age using dry lens weight, the lens weight (W) should be regressed on age (A) (Ishii 1975, Hagen *et al.* 1980, Zar 1984). Therefore, a predicted age $\hat{X}_i$ for a given lens weight Y_i and the confidence limits of $\hat{X}_i$ should be calculated on the basis of the procedure known as inverse prediction (Dapson 1980, Zar 1984). Using this procedure, the predicted $\hat{X}_i$ at a given Y_i is given by the equation

$$\hat{X}_i = \frac{Y_i - a}{b},$$

and the confidence limit L (L_u, the upper limit ; L_l, the lower limit) is calculated from the equation

$${L_u \brace L_l} = \bar{X} + \frac{b(Y_i - \bar{Y})}{K} \pm \frac{t}{K} \sqrt{S_{YX}^2 \left[\frac{(Y_i - \bar{Y})^2}{SS_X} + K \left(\frac{1}{m} + \frac{1}{n} \right) \right]}$$

where $\bar{X}$ = the mean of X, $\bar{Y}$ = the mean of Y, $K = b^2 - t^2 s_b^2$, s_b = the standard error of the regression coefficient, S_{YX}^2 = the residual mean square, SS_X = the sum of squared deviations of X, t = Student's t ($df = n-2$, $p = 0.05$), and n = sample size (Dapson 1980, Zar 1984). When $m = \infty$, L indicates the confidence limits of the mean prediction for the population, and when $m = 1$, L represents the confidence limits of the individual prediction (Dapson 1980). For the purposes of this study, X and L have been logarithmically transformed, so that 10^{X_i} gives the predicted age in days and 10^L gives its confidence limits.

In this study, data from both males and females were combined for the regression analysis since no significant difference was detected in the slope and elevation of the regression line between males and females. As for the figure showing the regression line with 95% confidence limits (see Fig. 1), we followed the presentation of Hagen *et al.* (1980). Although lens weight was regressed on age, we used the ordinate for the independent variable (age) and the abscissa for the dependent variable (lens weight) because the age was predicted by inverse prediction.

RESULTS AND DISCUSSION

The regression line and its 95% confidence limits for the individual predict-
ion are shown in Fig. 1.   The linear regression equation from our data in
Eothenomys smithii was proved to be

$$W = -1.415 + 2.131 \log_{10} A$$

and therefore a predicted age in days ($\hat{A}$) for a given lens weight in mg (W) was
given by the equation

$$\hat{A} = 10^{(W + 1.415)/2.131}$$

The slope and Y intercept of the regression line, and statistics necessary for
calculating the confidence limit are presented in Table 2.   Table 2 also
includes comparable information from the root vole, *Microtus oeconomus*
(Hagen *et al.* 1980), the feral house mouse, *M. m. molossinus* (Takada 1982a)
and the large Japanese field mouse, *Apodemus speciosus* (Takada 1982b).
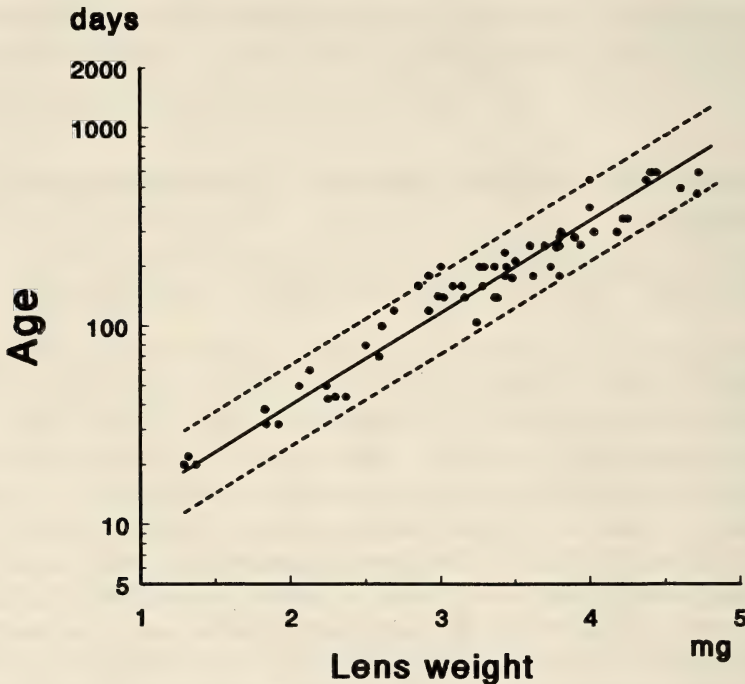
Fig. 1.   The relationship between the dry weight of the optic lenses (both the right and left
lenses combined) and age in days in 65 known-age Smith's red-backed voles, *Eothenomys
smithii*.   The solid line indicates the regression line, and broken lines its 95% confidence
limits for the individual prediction based on inverse prediction.   Note that the vertical axis
was used for the independent (age) variable, and horizontal axis for the dependent (lens
weight) variable, although lens weight was regressed on age.

Table 2. Statistics required for calculating the predicted ages and 95 % cofidence limits of the Smith's red-backed vole, *Eothenomys smithii*, the root vole, *Microtus oeconomus*, the feral house mouse, *Mus musculus molossinus*, and the large Japanese field mouse, *Apodemus speciosus*.

species	n	a	b	r	$\bar{X}$	$\bar{Y}$	S_{YX}	$S_{YX}/\bar{Y}$	SS_X	t
E. smithii This study	65	−1.415	2.131	0.971	2.177	3.223	0.213	0.0660	10.277	1.998
M. oeconomun Hagen *et al.* (1980)	31	−1.729	2.799	0.983	1.869	3.503	0.140	0.0400	2.336	2.045
M. m. molossinus Takada (1982a)	73	−2.954	4.13	0.976	1.994	5.281	0.358	0.0677	10.608	1.994
A. speciosus Takada (1982b)	14	−15.474	16.122	0.988	1.867	14.625	0.867	0.0549	1.230	2.179

n: sample size, a: Y intercept, b: slope, r: correlation coefficient, $\bar{X}$: mean of X, $\bar{Y}$: mean of Y, S_{YX}: standard error of estimate, SS_X: sum of squared deviations of X, t: Student's t ($d.f.=n-2$, $p=0.05$).

Dapson (1980) and Zar (1984) both recommended the presentation of $S_{YX}/\bar{Y}$ (the standard error of estimate (S_{YX}) divided by the mean of Y ($\bar{Y}$)), as an indicator for assessing the fitness of the regression and the accuracy of the technique. Smaller values of $S_{YX}/\bar{Y}$ indicate better fitness of the regression. The value of $S_{YX}/\bar{Y}$ for *E. smithii* (0.0660) is very close to that for *M. m. molossinus* (0.0677) (Takada 1982a), but larger than the values for either *M. oeconomus* (0.0400) (Hagen *et al.* 1980) or *A. speciosus* (0.0549) (Takada 1982b). When studies, which have used the regression analysis for age estimation, are compared (*e.g.*, those on *M. oeconomus* [Hagen *et al.* 1980], *M. m. molossinus* [Takada 1982a], *A. speciosus* [Takada 1982b], *S. floridanus* [Load 1959], *L. californicus* [Connolly *et al.* 1969], *L. arcticus* [Hearn and Mercer 1988] and *L. brachyurus* [Ando *et al.* 1992]), $S_{YX}/\bar{Y}$ is found to range from 0.0400 to 0.0823 (calculated by us). It is accordingly inferred that when the regression line fits the data well, $S_{YX}/\bar{Y}$ may be smaller than *ca.* 0.083 in small to medium-sized mammals such as rodents and lagomorphs. Since the value for *E. smithii* (0.0660) falls within the middle of this range, it can be said that our data from *E. smithii* fit the model equation (1) well.

Confidence intervals also indicate the accuracy of estimates derived from an age determination technique (Dapson 1980). Table 3 shows the predicted age ($\hat{A}$) at a given lens weight (W), its 95% confidence limits for the mean prediction and that for the individual prediction in *E. smithii*. The 95% confidence interval for the mean prediction at the mean of X (*i.e.*, $\bar{X}=2.1769$, the predicted age of 150 days) was calculated to be 17 days, occupying 11.3% of the predicted age (150 days). Similar figures have also been obtained for *M. oeconomus* (8.0%, Hagen *et al.* 1980), for *M. m. molossinus* (10%, Takada 1982a) and for *A. speciosus* (15%, Takada 1982b). In *E. smithii*, the 95% confidence interval (144 days) for the individual prediction, at the mean of X, occupied 96.0 % of the predicted age (150 days). The corresponding figure for *M. oeconomus* is 48 % (Hagen *et al.* 1980), for *M. m. molossinus* 83 % (Takada

Table 3. 95 percent confidence limits of predicted ages ($\hat{A}$) for the Smith's red-backed vole, *Eothenomys smithii*.

Lens weight W (mg)	Age $\hat{A}$ (days)	Mean predictions		Individual predictions	
		Lower age limit (days)	Upper age limit (days)	Lower age limit (days)	Upper age limit (days)
1.36	20	17	23	12	32
1.73	30	27	33	19	48
2.20	50	46	54	31	80
2.52	70	66	77	45	114
2.85	100	94	106	63	159
3.22	150	142	159	94	238
3.49	200	189	213	126	318
3.86	300	280	323	189	479
4.04	365	338	397	230	584
4.13	400	369	437	252	641
4.33	500	466	563	320	820
4.50	600	544	669	376	967

1982a) and for *A. speciosus* 55% (Takada 1982b). The 95% confidence interval for *E. smithii* was similar for the mean prediction, but was slightly broader for the individual prediction, when compared with the three other species. Although the confidence limits are influenced by various factors, such as sample size, the degree of dispersion of data, the mean of X and so on, increasing the sample size may be one possible way to improve the accuracy of age estimation of *E. smithii*.

The combined dry weights of both right and left lenses from 52 wild *E. smithii* captured on Mt. Wakasugi ranged from 1.95 to 4.49 mg (Ando unpublished data). From the equations defined above, a vole with a maximum lens weight of 4.49 mg would be estimated to be 591 days old, with the 95% confidence limits giving a range of 371 to 952 days for the individual prediction. About 80% of the voles (41/52) possessed lenses weighing below 4.04 mg indicating 365 days of a predicted age. Although there have been some field studies on population dynamics of *E. smithii* (Tanaka 1964, Igarashi 1980), no information has been available on the longevity of individuals in the wild, for instance, based on the capture-recapture method. Judging from the existence of voles with lenses weighing over 4.04 mg, it would appear, however, that some individuals in the wild could survive for over one year. In the laboratory, *E. smithii* has been known to live for more than three years (Ando *et al.* 1988). Field studies, in combination with the lens weight technique, are necessary in order to confirm the usefulness of the technique, especially in older voles.

Researchers on rodents have typically used wear of the tooth surface, and the length or the ratio of molar roots to determine age (Abe 1976 for *Clethrionomys rufocanus bedfordiae*, Hikida and Murakami 1980 for *Apodemus speciosus*, Alibhai 1980 for *Clethrionomys glareolus*). These methods, however,

can only be employed in rodents which have rooted molars, and Takada (1982a, 1982b) has already demonstrated that even in *M. m. molossinus* and *A. speciosus* which have rooted molars, the lens weight technique may be more reliable than the technique depending on the tooth wear. It should be emphasized, therefore, that the lens weight technique provides the best criterion for assessing age at present, particularly in rodents with rootless molars such as *E. smithii*.

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Postnatal growth, development and ultrasonic vocalization,of young Japanese field voles, *Microtus montebelli*

Yuko YOSHINAGA, Wakako OHNO and Satoshi SHIRAISHI

Zoological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka 812-81, Japan
Fax. +81-92-642-2804, e-mail. yyoshi @ agr. kyushu-u. ac. jp

Abstract.   Both postnatal growth and development of Japanese field voles, *Microtus montebelli*, were observed in a laboratory colony.   Details of the developmental aspects of the life-history of this species are described focusing on behavioral development including ultrasonic vocalization, sexual dimorphism and the use of sigmoidal models of growth patterns.   One purpose of the study was to provide a reliable basis for age-estimation of a wild population prior to conducting field investigations.   The overall pattern of development of *M. montebelli* was similar to that of other *Microtus* species, particularly in their relatively rapid development.   Young *M. montebelli* were found to vocalize intensively at an ultrasonic frequency of approximately 25 kHz until their eyes opened.   The Gompertz equation was selected from three nonlinear growth models (Gompertz, logistic and von Bertalanffy), as it best described the curves of body mass increase and of four external lengths, and it best estimated maximum growth rates derived from the Gompertz equations fitted to actual rates during a linear growth phase.   These features of the Gompertz equation seemed to be useful for analyzing growth patterns of wild voles. After 30 days, growth curves for each morphometric parameter diverged sexually, thus, weight-classes used for age estimation should differ between the sexes.

Key words : growth curve, *Microtus montebelli*, postnatal development, sexual dimorphism, ultrasonic vocalization.

Microtus voles grow relatively rapidly when compared with other muroid rodents (Zullinger *et al.* 1984, Dewsbury 1990), although they appear to show considerable interspecific variation in rates of postnatal development even under similar laboratory conditions (Nadeau 1985, Innes and Millar 1994).   It is suggested that among *Microtus* species, some aspects of interspecific variation in postnatal development correlate with their type of social organization, *i.e.,* monogamous species tend to develop physically and behaviorally more slowly than polygamous species (Kleiman 1977, McGuire and Novak 1984, 1986, Dewsbury 1990).   To make such a correlation clear, data on development as well as on mating systems are required from a substantial number of animals from within a restricted taxonomic group, yet from a group that shows a

diversity of ecological adaptations. The genus *Microtus* offers an excellent opportunity for this.

Physical development, growth and the reproductive patterns of the Japanese, field vole, *M. montebelli*, are well documented (Shiraishi 1969, Miyao 1974, Obara 1975, Kudo and Oki 1982). Since variability in these characteristics may exist among local populations of a given species, it is necessary to collect all such data from voles from a single targeted population. Furthermore, for *M. montebelli* little information has been reported on the subjects of fitting sigmoidal models to growth patterns on the appearance of sexual dimorphism, behavioral development or on ultrasonic vocalization by infants. The latter is of particular interest given that interspecific variation in ultrasonic vocalization has recently been the focus of correlations with social systems (B. H. Blake personal communication).

Field researchers often assign captured voles to age-classes (typically juvenile, subadult and adult) on the basis of their weight when caught. As voles from different localities may differ in body size (Bondrup-Nielsen and Ims 1990), it is important to confirm how old the voles in each age-class are on the basis of the weights of known-age voles from the same study population. Moreover, this may be an effective mean of making more detailed age-estimation and growth analyses by restoring original growth curves from sporadic field data. With this aim in mind, it is important to test statistically the effectiveness of fit of growth models using known-age samples.

Our primary aim, therefore, in writing this paper is to describe the details of the developmental aspects of *M. montebelli*, focusing on fitting various models to growth patterns, and to describe the appearance of sexual dimorphism and behavioral development including ultrasonic vocalization, as components of the species' life-history. Our secondary aim is to provide a reliable basis for age-estimation of a vole population at our study site prior to conducting field investigations.

MATERIALS AND METHODS

The captive breeding colony of voles used in this study was derived from wild-caught *M. montebelli* from a meadow on the northern rim of Mt. Aso, Kumamoto Prefecture, Japan. Pairs of voles were housed in stainless steel cages ($20 \times 25 \times 43$ cm) with chambers containing straw and cotton wool as nesting material. The growth patterns of their infants (*i.e.,* of first generation laboratory-born voles) were observed. The room in which the colony was housed was maintained at 22 ± 2 °C with a 14 hour light and 10 hour dark photoperiod (the lights were switched on each day at 08 : 00). A commercial herbivore diet (ZF, Oriental Yeast, Tokyo) and water were provided *ad libitum* with an occasional supplement of sweet potato. Newborn voles were left with their parents from birth (day 0) until 20 days old, when they were removed and housed together with their litter mates until approximately 60 days old. Thereafter, males and females were housed separately.

Fourteen male and 14 female *M. montebelli* from seven litters were observed closely from birth until day 20. Each infant was removed from its natal nest, placed on to a 50 cm diameter glass tray and its behaviour was monitored for two minutes each day using an 8-mm video recorder (CCD–V800, Sony, Tokyo) connected to a Mini–2 bat detector (Ultra Sound Advice, London, UK) set to 25 kHz. This frequency was selected as the frequency at which infant vocalizations were most easily detected, after preliminary tests made at intervals of 5 kHz. Because ultrasonic vocalizations were likely to change in their duration over time in the preliminary tests, we noted whether vocalizations were sustained for more than one minute (continuous vocalization) or not (discontinuous vocalization), that is more than half of the two minute observation periods. Physical development was also observed, and obvious changes in the eyes, ears, digits, incisors and pelage were recorded individually.

Ten males and 10 females from five litters were used as subjects for measurements of five variables. These were: body length (from snout tip to anus), tail length (from anus to tail tip), hind foot length (without claw), ear length and body mass. These measurements were made every second day from day 0 to day 20, every 5 days to day 50, then every 10 days to day 150. Weight was measured to the nearest 0.01 g on an electronic balance (PJ3000, Mettler, Switzerland), and length was measured to the nearest 0.1 mm with a ruler or vernier callipers. To compensate for the reduction in sample size caused by deaths before weaning, additional growth data were obtained after day 20 from another litter consisting of one male and four females.

Growth curves were fitted with non-linear regression models using iterative least squares (Zullinger *et al.* 1984). Three sigmoidal equations were used in this study:
the Gompertz equation,

$$M(t) = A \times e^{-e^{-K(t-I)}}$$

the logistic equation,

$$M(t) = A\{e^{-K(t-I)} + 1\}^{-1}$$

and the von Bertalanffy equation,

$$M(t) = A\{1 - 1/3 \ e^{-K(t-I)}\}^3$$

where $M(t)$ = mass (g) or length (mm) at age t, A = asymptotic value, K = growth rate constant (day^{-1}), and I = age (days) at the inflection point (Ricklefs 1967).

The abilities of these three equations to fit the growth data were compared in relation to: correlation coefficients, coefficients of variation in estimated parameters (A, K, and I), and the residual sum of squares. To compare rates of early growth between males and females, we also calculated simple linear regression equations for mass and lengths over a linear growth phase. A linear growth phase was defined as a period when mass or length increased relatively constantly each day. Sexual differences reflected by regression

equations were tested using the t-test following Zar (1984). Maximum growth rates at inflection points (MGR_E) were estimated from the parameters of the best fit equations (Ricklefs 1967), and were compared with the observed maximum increases of mass and length per day (MGR_O) and the regression coefficients (b), in order to evaluate the usefulness of growth parameters estimated by curve fitting. The formulae for MGR_E were as follows:
for the Gompertz equation,

$$MGR_E = K \times A \times 1/e$$

for the logistic equation,

$$MGR_E = K \times A \times 1/2$$

and for the von Bertalanffy equation,

$$MGR_E = K \times A \times 8/27$$

The statistical significance of differences between the sexes was tested using an unpaired two-tailed t-test for the age at which developmental events occurred. All statistical analyses follow Zar (1984). Means are expressed plus or minus one standard deviation.

RESULTS

1. Physical development
Neonates were essentially naked, but with short pale hairs and pigmentation just detectable on their backs. Neonates had attached digits, folded ear pinnae and eyelids and lacked erupted teeth. As they grew, their hair gradually became denser, their ear pinnae unfolded (day 2.5 ± 0.6), their digits separated, their incisors erupted and their auditory meatus (day 7.3 ± 0.6) and their eyes (day 8.3 ± 0.9) opened. Each event occurred within a range of 1–3 days, and no sexual differences were observed (t values ranged from 0.20 to 2.05, all $p>0.05$). In females, teats became noticeable at day 1.7 ± 1.1. To identify the order of digit separation, digits were numbered one to five from the innermost digit, the first digits in the fore foot were, however, invisible externally.

The outermost digits separated first with digits 4–5 in the fore foot separating on day 3.9 ± 0.7, and digits 4–5 in the hind foot separating on day 4.8 ± 0.7, $n=28$), then the innermost digits separated, digits 2–3 in the fore foot on day 5.6 ± 0.7, and digits 1–2 in the hind foot on day 5.1 ± 0.9), and finally the remaining digits became separated (3–4 on the fore foot on day 6.8 ± 0.6 and 2–3 on the hind foot on day 7.4 ± 0.8 and 3–4 on the hind foot on day 7.4 ± 0.8). Each fore foot digit separated significantly earlier than its hind foot homologue (t values ranged from 2.86 to 8.63, $p<0.01$). The lower incisors (day 5.6 ± 0.6) erupted before the upper incisors (day 5.9 ± 0.7) in every individual. All individuals completed their external development by day 10, by which time their eyes had opened and their juvenile pelage was complete (day 9.6 ± 0.7). Pups ingested solid food from day 9 or 10 onwards (day 9.3 ± 0.5, $n=28$) and this did not differ

between males and females ($t=0.39$, $p=0.699$).

2. Behavioral development

Nine activities were distinguishable during the daily two minute observation periods. These were: resting, rolling over, pivoting, crawling, walking unsteadily, normal walking, moving backwards, grooming and standing up. Of these nine, the last three were relatively capricious and their developmental stages were not so clear. Rolling over, pivoting, crawling and unsteady walking were specific to younger voles, and mobility developed in this order (Fig. 1a, b). Neonates were entirely immobile. They were unsteady even while resting and 70% of them rolled over.
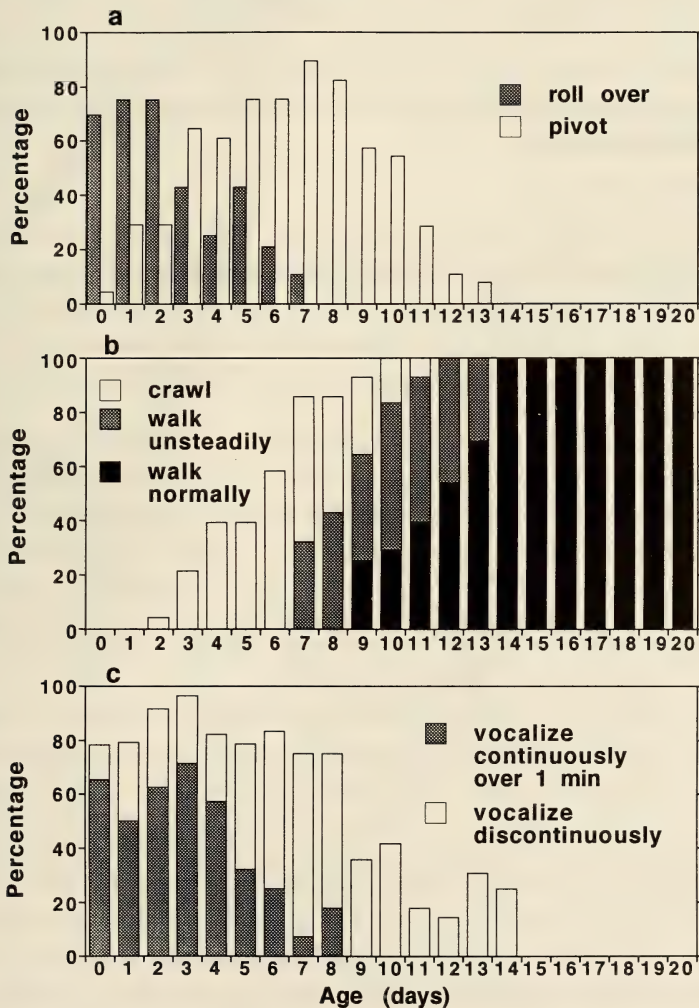
Fig. 1. Behavioral development of infant Japanese field voles for the first 20 days ($n=28$). Each column indicates the percentage of individuals which showed behaviors specific to a younger stage, a) rolling over and pivoting, b) moving forward, c) ultrasonic vocalizations during the two minute observation.

The age at which each activity commenced or disappeared ranged from 3–10 days, and this did not differ between the sexes (t values ranged from 0.19 to 0.83, $p > 0.4$). After day 14, all individuals were able to walk normally, and by this age they were adult-like in all their activities.

3. Ultrasonic vocalization

Ultrasonic vocalizations at around 25 kHz were first emitted as early as day 0.5 ± 0.9 (days 0–3). Continuous vocalization was recorded for 65% of all neonates (Fig. 1c). Young voles emitted ultrasound especially when they were rolling over, and most individuals (75–96% of examinations) vocalized until day 8, when their eyes opened. Continuous vocalizing was last recorded on day 5.1 ± 2.1 (days 0–8). After day 9, all vocalizing became discontinuous and fewer than 50% of young voles vocalized at 25 kHz, although some pups continued to vocalize until day 14 (day 10.3 ± 2.1 on average). It was clear, therefore, that the incidence and duration of ultrasonic vocalizations among neonates changed once their eyes had opened.

4. Growth curves

The body size of neonate males and females was similar. Neonate males weighed 2.99 ± 0.38 g ($n = 10$) and neonate females ($n = 10$) weighed 2.83 ± 0.44 g ($t = 0.87$, $p = 0.394$), body lengths were 38.5 ± 2.3 mm for males and 37.7 ± 2.9 mm for females ($t = 0.69$, $p = 0.502$), tail lengths were 8.4 ± 0.7 mm and 8.6 ± 0.9 mm ($t = 0.78$, $p = 0.448$), and hind foot lengths were 6.6 ± 0.5 mm and 6.4 ± 0.4 mm respectively ($t = 0.91$, $p = 0.375$).

Body mass increased almost continuously from birth for the first 90 days for males, and for the first 60 days for females (Fig. 2, upper graph). For the first 30 days the growth curves of male and female body mass did not differ (Fig. 2), however the slopes of male (0.74 g/day, $r^2 = 0.995$, $F = 2145.9$, $p = 0.0001$) and female (0.69 g/day, $r^2 = 0.996$, $F = 2646.1$, $p = 0.0001$) regression lines differed significantly ($t = 2.27$, $p = 0.034$). Thereafter, growth rates of males were generally greater than those of females (Fig. 2, lower graph) and the growth curves of males and females continued to diverge (Fig. 2, upper graph).

Male and female body length increased linearly for the first 14 days (Fig. 3, lower graph) with the slopes of male and female regression lines not differing significantly ($t = 0.54$, $p = 0.600$). The common regression coefficient was 2.9 mm/day ($r^2 = 0.995$, $F = 2888.2$, $p = 0.0001$). Similarly, increases in tail length over the first 20 days ($t = 0.56$, $p = 0.585$, Fig. 4, lower graph) in hind foot length over the first 10 days ($t = 0.48$, $p = 0.646$, Fig. 5, lower graph) and in ear length over the first 14 days ($t = 0.93$, $p = 0.369$, Fig. 6, lower graph) were all judged to be linear with regression line slopes that did not differ between males and females. The common regression coefficients for males and females, of tail, hind foot and ear lengths were 1.4 mm/day ($r^2 = 0.995$, $F = 4383.7$, $p = 0.0001$), 0.9 mm/day ($r^2 = 0.995$, $F = 1929.5$, $p = 0.0001$) and 0.5 mm/day ($r^2 = 0.989$, $F = 1215.0$, $p = 0.0001$), respectively. Thus, it was apparent that during the linear growth phases, differences in body, tail, hind foot and ear lengths between malen and
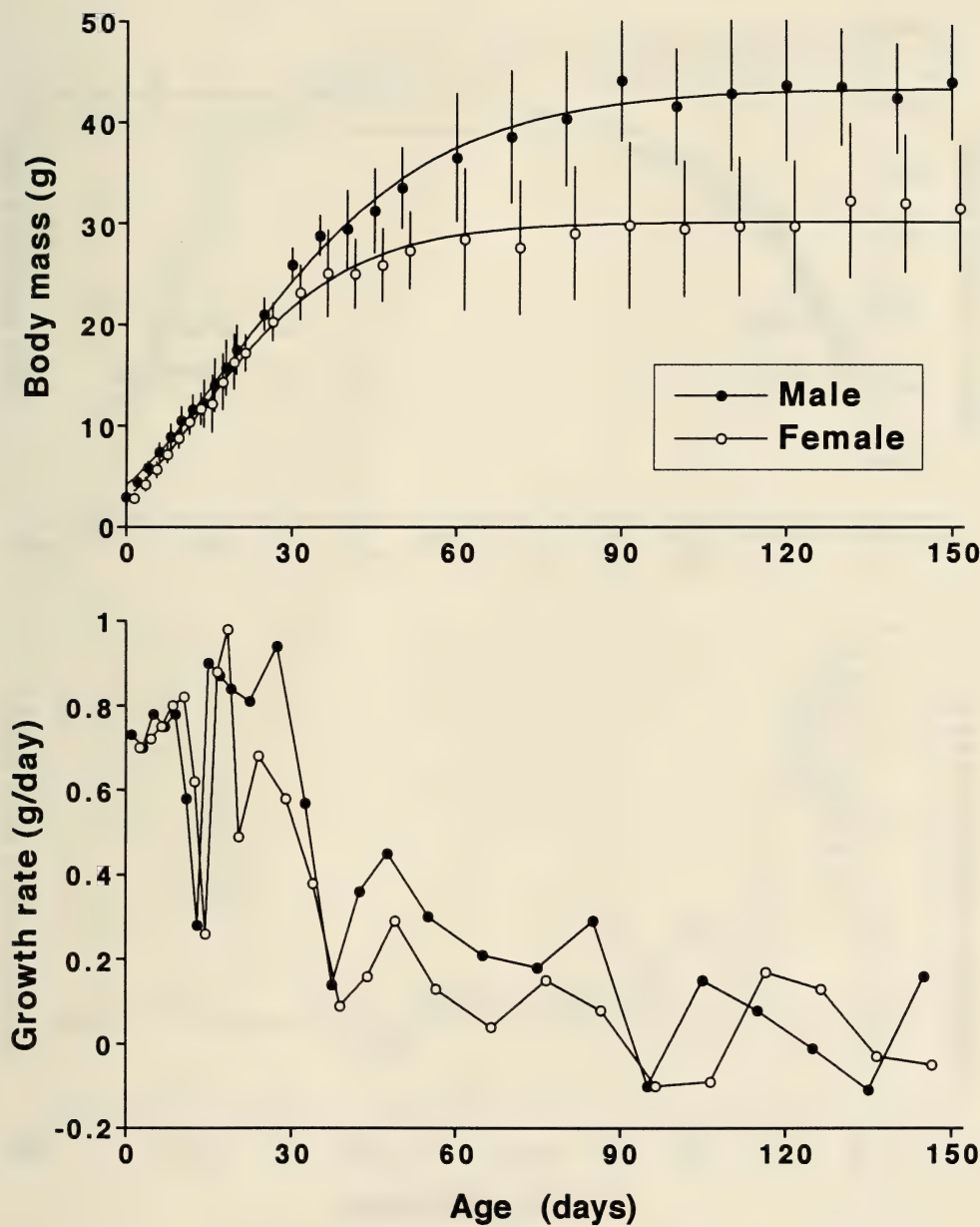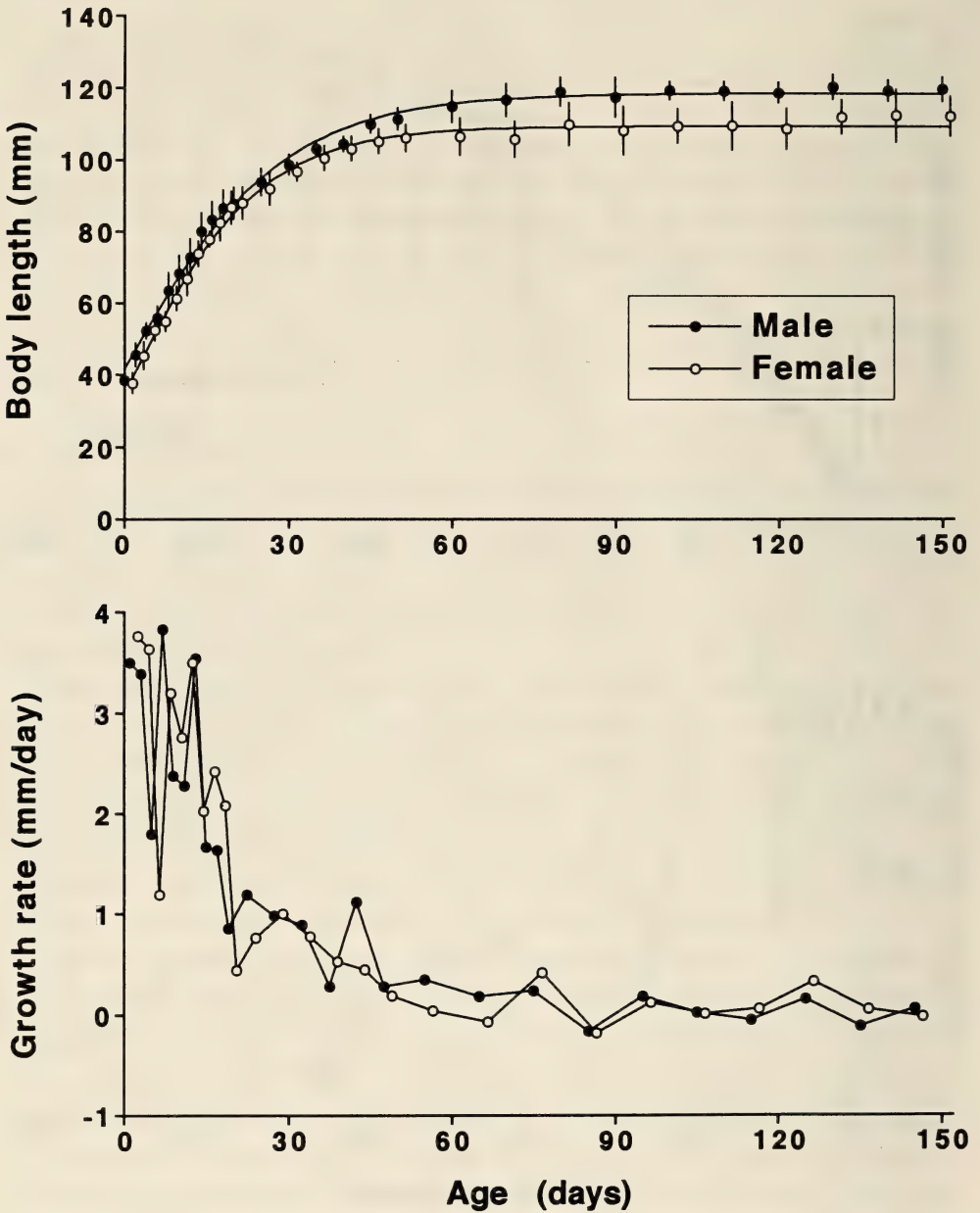
Fig. 2. Gompertz plots for postnatal mean body mass (upper graph) and growth rates per day (lower graph) against age in the Japanese field vole. Actual data points are represented by solid circles (male, $n=10$) and open circles (female, $n=10$). Vertical bars indicate $\pm$ 1 *SD*. Growth parameters are found in Table 1.
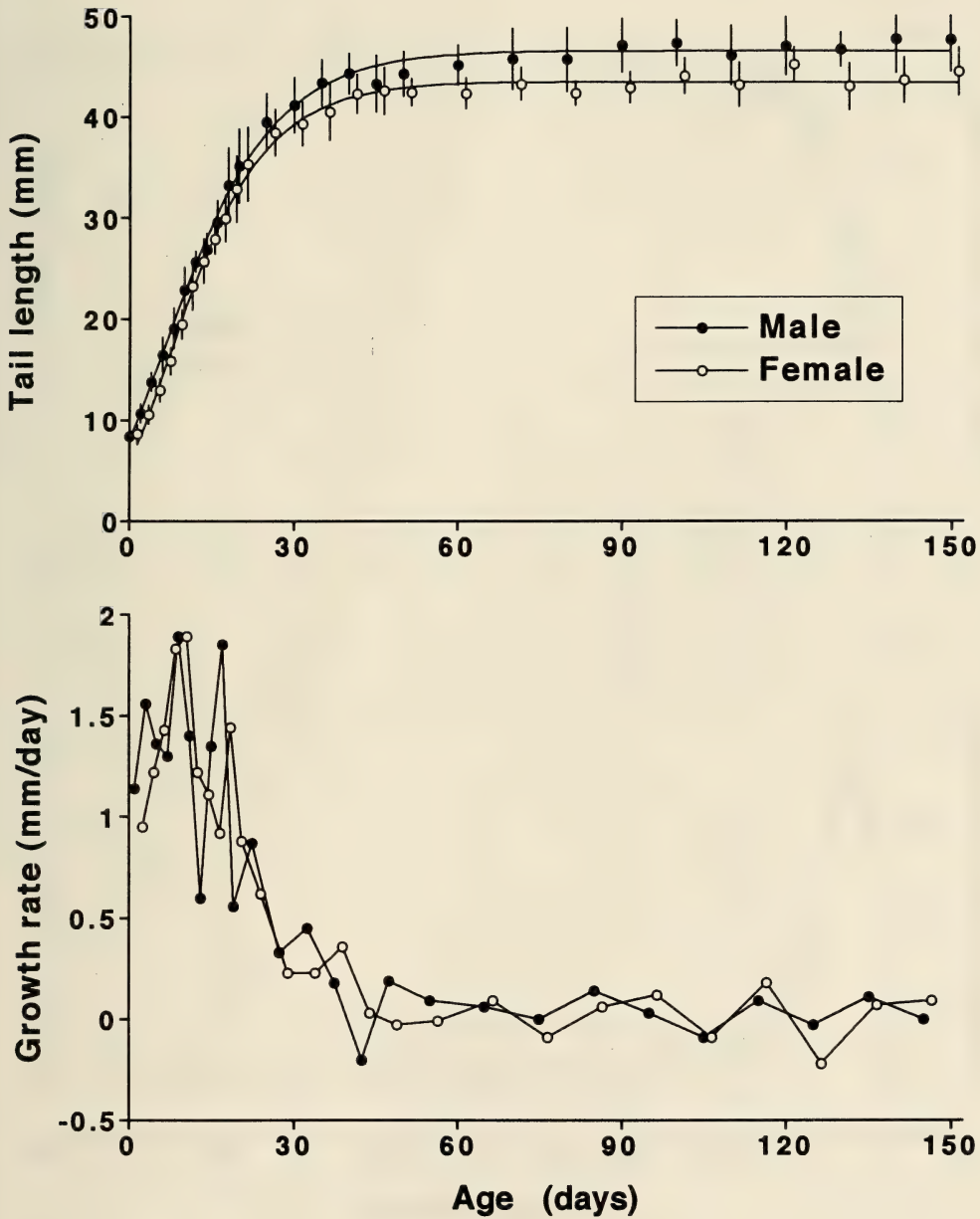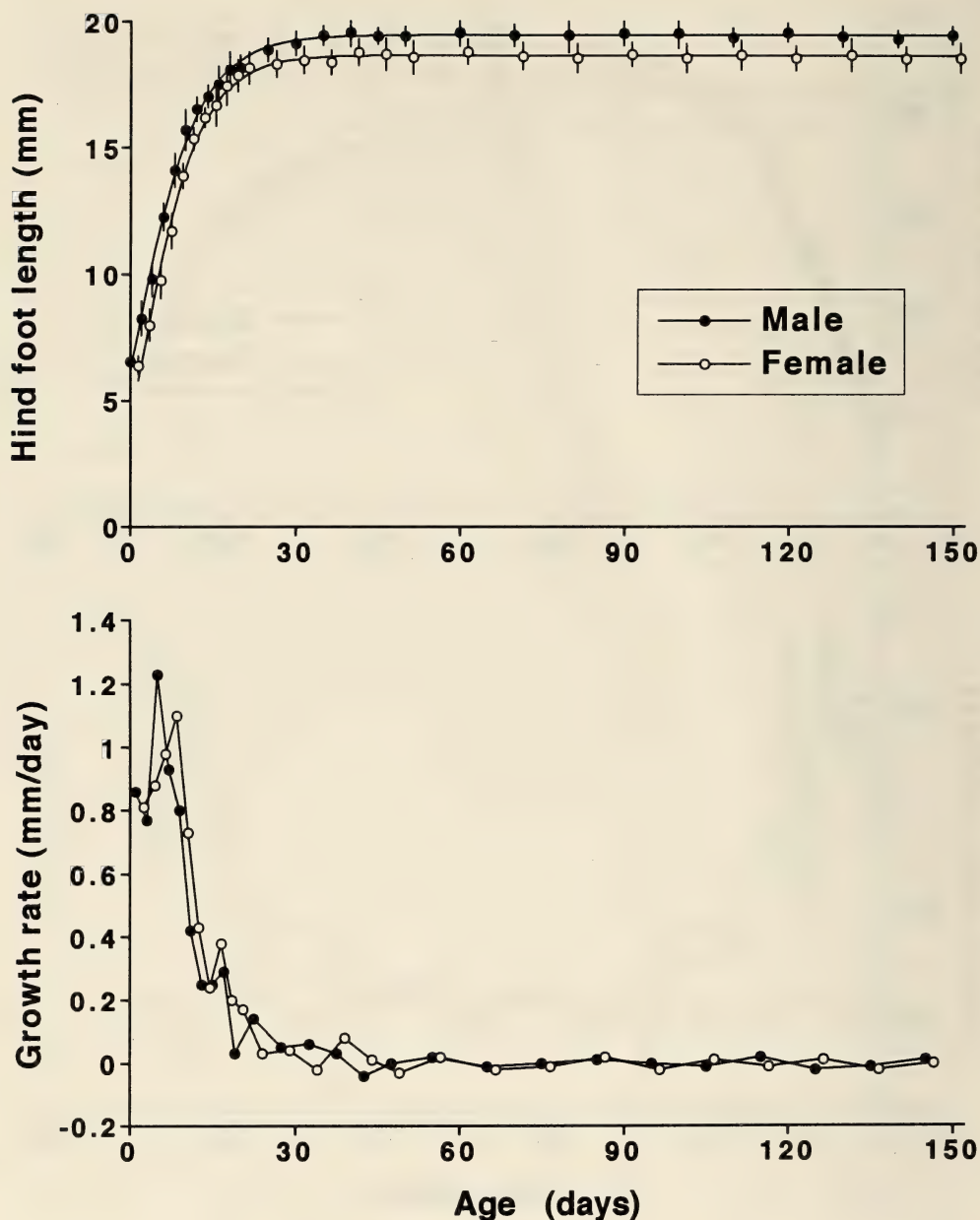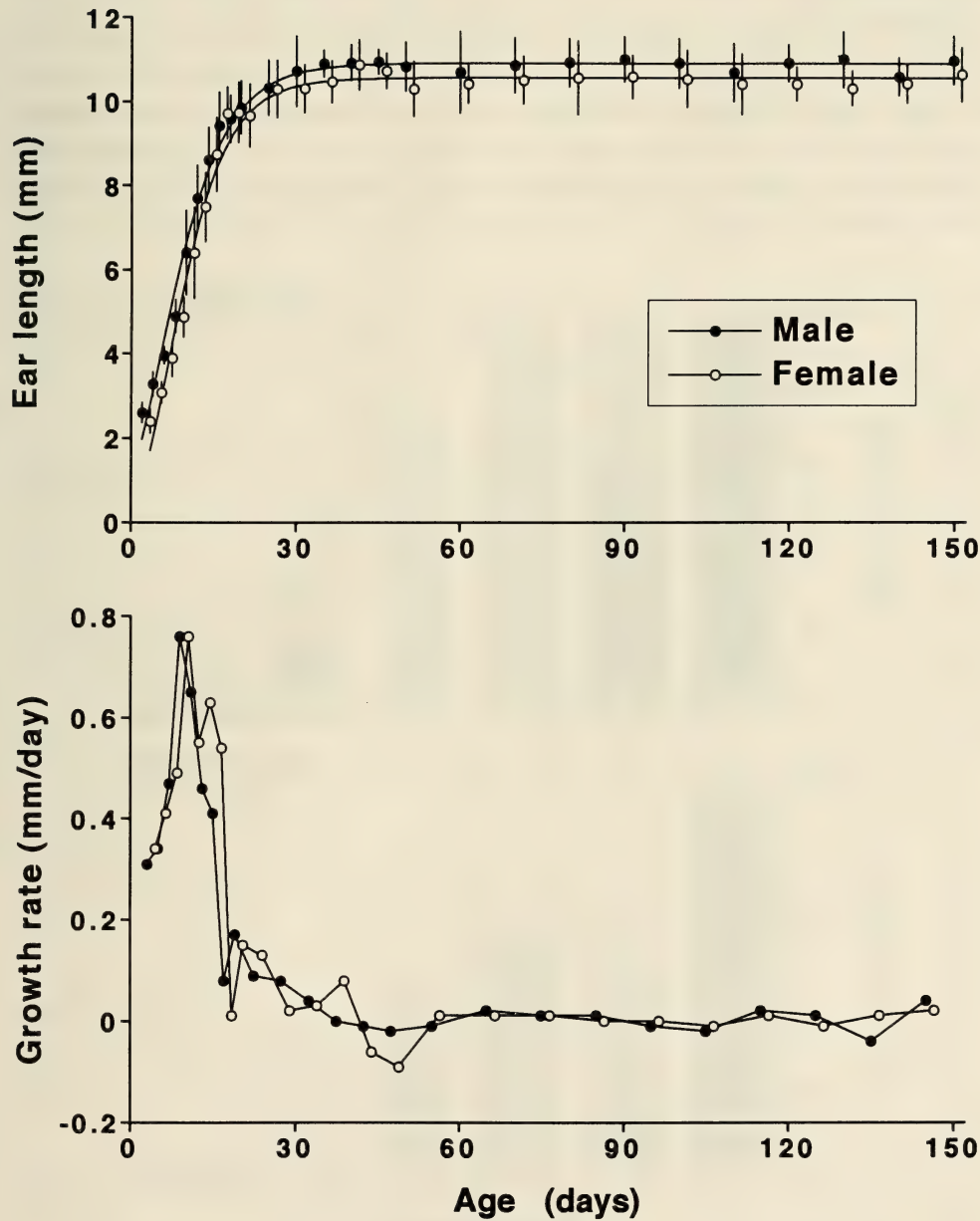
Fig. 3. Gompertz plots for postnatal mean body length (upper graph) and growth rates per day (lower graph) against age in the Japanese field vole.   Actual data points are represented by solid circles (male, *n*=10) and open circles (female, *n*=10).   Vertical bars indicate ± 1 *SD*. Growth parameters are found in Table 1.

Fig. 4. Gompertz plots for postnatal mean tail length (upper graph) and growth rates per day (lower graph) against age in the Japanese field vole. Actual data points are represented by solid circles (male, $n=10$) and open circles (female, $n=10$). Vertical bars indicate ± 1 *SD*. Growth parameters are found in Table 1.

Fig. 5. Gompertz plots for postnatal mean hind foot length (upper graph) and growth rates per day (lower graph) against age in the Japanese field vole. Actual data points are represented by solid circles (male, $n=10$) and open circles (female, $n=10$). Vertical bars indicate $\pm$ 1 *SD*. Growth parameters are found in Table 1.

Fig. 6. Gompertz plots for postnatal mean ear length (upper graph) and growth rates per day (lower graph) against age in the Japanese field vole. Actula data points are represented by solid circles (male, $n=10$) and open circles (female, $n=10$). Vertical bars indicate $\pm$ 1 *SD*. Growth parameters are found in Table 1.

females were not detectable, however at about day 30 the growth curves of all lengths diverged sexually (Figs. 3-6, upper graphs).

Although body mass showed considerable variation (Fig. 2, upper graph), we were able to assign individual voles to one of three age-classes on the basis of body mass (Fig. 7). Among males, 85% of individuals which weighed 15-25 g ($n=33$) were younger than 30 days, 94% of individuals which weighed 25-35 g ($n=51$) were 30 to 90 days old, and 69% of individuals weighing over 35 g ($n=86$) were more than 90 days old (Fig. 7). Among females, which were lighter than males, 93% of those weighing 15-20 g ($n=28$) were less than 30 days old, 61% of those weighing 20-30 g ($n=93$) were 30-90 days old, and 67% of those weighing over 30 g ($n=39$) were more than 90 days old (Fig. 7).
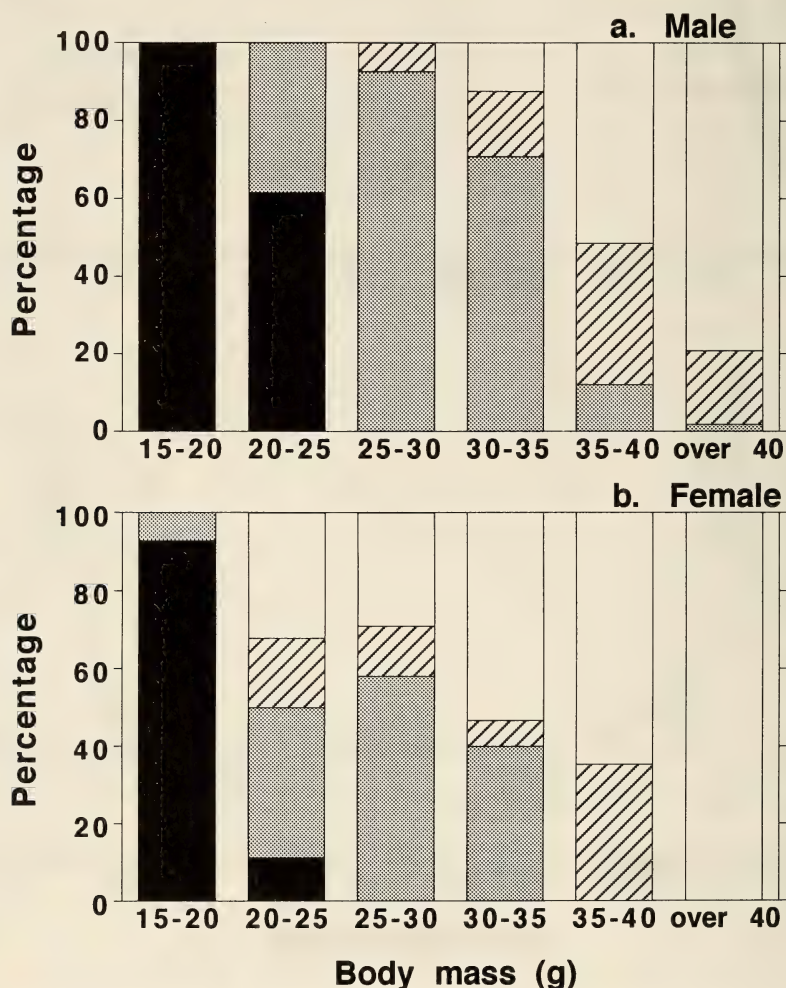
Fig. 7. The relationship between weight-classes and age-classes. Each colums indicates the percentage of individuals under 30 days (■), 30-60 days (□), 60-90 days (▨) over 90 days of age (□) in a) males and b) females in each weight class at intervals of 5 g.

5. Models for postnatal growth

Data on postnatal growth were evaluated based on three non-linear models (Gompertz, logistic and von Bertalanffy equations, parameter estimates for the best fit equations are summarized in Table 1). The predicted values had correlation coefficients over 0.99 in all cases (Table 1). Because of these high correlations, it was difficult to distinguish graphically among the three models, however, after deriving an equation based on each growth model, we chose the Gompertz equation on the basis of the statistical characteristics of the parameter estimates.

For all three models, the model showing the lowest residual sum of squares varied with each parameter, for example, the von Bertalanffy equation was

Table 1. Growth parameters in the Japanese field vole, *M. montebelli*, derived from the Gompertz, logistic and von Bertalanffy equations.

	Model	Sex	Residual sum of squares	r	Asymptote Estimate*	Coefficient of variation (%)	Growth rate constant Estimate (days⁻¹)	Coefficient of variation (%)	Inflection point Estimate (days)	Coefficient of variation (%)
Body mass	Gompertz	M	20.93	0.998	43.30	0.88	0.0463	3.38	18.3	2.39
		F	20.36	0.996	30.12	1.02	0.0655	4.89	11.5	3.80
	Logistic	M	53.11	0.995	42.50	1.26	0.0684	5.14	27.0	3.14
		F	35.40	0.993	29.70	1.27	0.0953	6.57	17.5	3.84
	von Bertalanffy	M	16.77	0.999	43.78	0.84	0.0392	3.11	13.9	2.56
		F	17.33	0.996	30.33	0.97	0.0562	4.49	8.4	4.64
Body length	Gompertz	M	84.84	0.998	118.60	0.52	0.0621	3.40	0.8	41.76
		F	73.84	0.997	109.43	0.49	0.0759	3.48	0.4	71.55
	Logitic	M	174.37	0.995	117.90	0.72	0.0783	4.88	6.7	6.56
		F	115.01	0.996	108.85	0.59	0.0964	4.37	5.3	6.40
	von Bertalanffy	M	61.45	0.998	118.90	0.45	0.0569	2.90	-1.7	18.73
		F	65.85	0.998	109.66	0.47	0.0695	3.28	-1.7	19.50
Tail length	Gompertz	M	16.39	0.998	46.50	0.53	0.0880	3.05	6.4	3.36
		F	13.34	0.998	43.39	0.49	0.0997	2.97	5.6	3.44
	Logistic	M	24.64	0.997	46.12	0.62	0.1232	3.87	10.9	2.48
		F	16.83	0.998	43.08	0.53	0.1384	3.44	9.6	2.27
	von Bertalanffy	M	17.99	0.998	46.68	0.56	0.0772	3.15	4.1	5.60
		F	16.65	0.998	43.53	0.56	0.0876	3.27	3.6	6.18
Hind foot length	Gompertz	M	1.20	0.998	19.46	0.29	0.1546	2.50	0.9	13.95
		F	1.36	0.998	18.67	0.32	0.1687	2.81	0.9	14.02
	Logistic	M	1.36	0.998	19.40	0.31	0.1965	2.73	3.4	3.61
		F	0.57	0.999	18.61	0.20	0.2149	1.87	3.3	2.38
	von Gertalanffy	M	1.48	0.998	19.49	0.33	0.1413	2.75	-0.2	75.61
		F	1.97	0.997	18.69	0.39	0.1541	3.35	-0.1	164.45
Ear length	Gompertz	M	1.62	0.995	10.89	0.64	0.1545	4.53	5.5	3.89
		F	2.34	0.993	10.54	0.78	0.1726	5.62	5.5	4.42
	Logistic	M	0.82	0.998	10.84	0.44	0.2130	3.33	8.2	1.81
		F	1.18	0.996	10.50	0.54	0.2401	4.16	8.0	2.13
	von Bertalanffy	M	2.24	0.994	10.91	0.76	0.1362	5.23	4.1	6.24
		F	3.10	0.991	10.56	0.90	0.1515	6.35	4.2	6.75

*Weight in g and length in mm.

lowest for body mass and length, the Gompertz equation was lowest for tail length, the logistic equation was lowest for ear length, and the Gompertz and logistic equations were lowest for male and female hind foot length, respectively (Table 1).   The coefficients of variation (a measure of the variation that each parameter exhibits, and the reliability of each parameter without affecting the overall predictive capability of the model) for the estimates of growth parameters of asymptotic values (A) and growth rate constants (K) were consistently less, when derived from the model yielding the lowest residual sum of squares (Table 1).   When considering inflection points I, the lowest coefficient of variation was obtained from the logistic equation for all measurements except for body mass.   For each approximation where the logistic or von Bertalanffy equation resulted in the lowest residual sum of squares, and the lowest coefficient of variation in A and K, the next best approximation was always provided by the Gompertz model.   For these two criteria, the logistic equation provided the worst approximation for body mass and length of the three models, while the von Bertalanffy equation provided the worst approximation of hind foot and ear lengths.   Thus the Gompertz model was chosen as the best compromise for approximating all growth curves for *M. montebelli*, since even those measures best fit by either the logistic or von Bertalanffy models also fitted reasonably well with the Gompertz model.   The lines in the upper graphs of Figs. 2–6, portray growth curves predicted by Gompertz equations.

When comparing the estimated maximum growth rates (MGR_E) derived from fitted Gompertz, logistic and von Bertalanffy equations, a consistent pattern was found in the relative magnitudes of their values (Table 2).   The MGR_E value was greatest with the logistic equation and least with the von Bertalanffy equation.   Since MGR_E values derived from the logistic equation were consistently greater than either the observed maximum growth rates

Table 2.   Comparisons among observed maximum growth rates (MGR_O) estimated maximum growth rates derived from three sigmoidal models (MGR_E) and regression coefficients during linear growth phases (b) in five measurements.

Measure	Sex	MGR_O	b	MGR_E		
				Gompertz[1]	logistic[2]	von Bertalanffy[3]
Body mass (g/day)	M	0.94	0.74	0.74	1.45	0.51
	F	0.98	0.69	0.73	1.42	0.51
Body length (mm/day)	M	3.83	2.87	2.71	4.62	2.00
	F	3.76	2.81	3.06	5.24	2.26
Tail length (mm/day)	M	1.89	1.36	1.51	2.84	1.07
	F	1.89	1.38	1.59	2.98	1.13
Hind foot length (mm/day)	M	1.23	0.94	1.11	1.91	0.82
	F	1.10	0.92	1.16	2.00	0.85
Ear length (mm/day)	M	0.76	0.52	0.62	1.15	0.44
	F	0.76	0.55	0.67	1.26	0.47

[1] $MGR_E = K \times A \times 1/e$, [2] $MGR_E = K \times A \times 1/2$, [3] $MGR_E = K \times A \times 8/27$

(MGR_O) or the slopes of regression lines during the linear growth phase (b), those values were considered likely to overestimate growth rates during rapid growth phases (Table 2). Conversely, MGR_E values from the von Bertalanffy equation were consistently lower than either MGR_O or b values. MGR_E values obtained from the Gompertz equation were closest to MGR_O and b values of these three equations in most cases. Thus, the Gompertz equation was again selected as the best model for approximating the growth rates of *M. montebelli*.

When comparing the estimated maximum growth rates (MGR_E) from fitted Gompertz equations and observed maximum growth rates (MGR_O) and regression coefficients (b), MGR_O values were higher than the other two values in almost all cases. MGR_E tended to approximate b, which reflects average growth rates during the linear growth phase (Table 2). Observed growth rates fluctuated considerably even when growth seemed to be more linear (Figs. 2–6, lower graphs), so it is suggested that MGR_E values derived from the Gompertz equation are a good indication of average growth rates during linear growth phases.

DISCUSSION

The overall patterns of physical and behavioral development of the Japanese field vole, *M. montebelli*, fall within the ranges exhibited by other *Microtus* species (Pepin and Baron 1978, Nadeau 1985). The growth rate of mass, 0.7 g/day, calculated as the slope of simple regression line places this species within a group with moderate growth rates among the 15 other species of *Microtus* reviewed by Innes and Millar (1994). Innes and Millar (1994) also found, however, significant positive correlations among *Microtus* species between female weight and certain other traits, such as litter size, neonate weight and growth rate to weaning. Thus, interspecific comparisons of growth rates should be made after growth rates have been standardized by female weights.

When standardized growth rates (weight increase per day as a percentage of female weight) are compared among 13 *Microtus* species, *M. montebelli* (2.33 % per day, this study) ranks as the second most rapidly growing species (others range from 0.94% to 3.14% per day, calculated from Innes and Millar's [1994] data). Although few data relating to postnatal development are available for comparison with this study, the age at which eyes open has been reported for a number of *Microtus* species, and is used as an index of maturation (Dewsbury 1990). The eyes of *M. montebelli* open earlier (day 8.3) than in either *M. ochrogaster*, *M. pinetorum*, *M. montanus* or *M. pennsylvanicus* (days 9.1–11.7, Dewsbury 1990). Thus, it seems that *M. montebelli* belongs to a rapidly developing sub-group of *Microtus* species. Kleiman (1977) considered that a long period of maturation for young voles was a characteristic of monogamy, suggesting, therefore, that the rapid growth pattern of young *M. montebelli* may be related to non-monogamous traits.

According to Glucksmann (1974), sexually dimorphic animals are unlikely

to exhibit sexual differences until puberty. Young *M. montebelli* certainly showed no sexual differences in physical development (completed by day 10), or in the processes of behavioral development (completed by day 14), and until about day 30, the growth curves of body mass and of four external measurements were indistinguishable between males and females. After 30 days the growth curves of males and females diverged clearly, and males became larger than females. Even during the linear growth phase, rates of growth of body mass differed sexually, although other measurements did not.

A reasonable explanation for male-biased sexual dimorphism among microtine voles has been made only in relation to types of mating systems (Heske and Ostfeld 1990, Boonstra *et al.* 1993). Through interspecific comparisons among *Microtus* species, the ratios of male to female body masses fall roughly into three groups corresponding to their mating systems : 1.0 for monogamous species, 1.2 for promiscuous species and 1.3 for polygynous species (Yoshinaga *et al.* 1997b). Thus, the apparent male-biased sexual dimorphism in *M. montebelli* seems to indicate that they may be polygynous. Observations appear to support this in as much as during the breeding season, resident male wild *M. montebelli* maintain intra-sexual exclusive home ranges which overlap with those of several females (Yoshinaga unpublished data). There appear to be, however, several discrepancies in previous reports on the correlation between degrees of sexual dimorphism and mating systems in microtine species (Dewsbury *et al.* 1980, Boonstra *et al.* 1993, Ostfeld and Heske 1993), indicating that more detailed and more reliable data on development and mating systems among voles are necessary in order to discuss more effectively underlying theories explaining such correlations.

For age-estimation in the field, voles could be assigned to three age-classes, *i.e.*, juveniles (voles younger than 30 days), subadults (30 to 90 days) and adults (older than 90 days), on the basis of growth in body mass data from the laboratory colony. Since *M. montebelli* is sexually dimorphic, weight criteria for each age-class should differ between males and females. For males, for example, voles weighing 15–25 g should be considered juvenile, those weighing 25–35 g should be considered subadult, and those weighing over 35 g adults, whereas for females, the weight criteria for each age-class should be 5 g lighter than in males. These age-weight classes should be applicable for field studies of *M. montebelli* in our region.

Moreover, more detailed age-estimation and growth analyses may be effectively achieved by restoring original growth curves from sporadic field data. During most field studies, rates of weight increase are only available between consecutive captures. Since growth curves of many mammals are sigmoid (Zullinger *et al.* 1984), the relationship between weight and weight increase should theoretically follow a differentiated sigmoidal equation. For modeling growth patterns of *M. montebelli*, the Gompertz equation was selected from three sigmoidal models tested statistically in this study. The differentiated Gompertz equation has also been demonstrated to fit a data set of weights and weight increases collected from wild voles (Yoshinaga *et al.* 1997a).

Restored growth curves of wild voles have asymptotic weights which differ according to the month of birth (Yoshinaga *et al*. 1997a), thus it may be of great value to use the generated growth curves for age-estimation of captured voles.

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Acquisition of food begging behavior by red foxes in the Shiretoko National Park, Hokkaido, Japan

Hideharu Tsukada

Regional Sciences, Faculty of Letters, Hokkaido University, N10 W7 Kita-ku, Sapporo 060, Japan
(Present address : Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 Kita-ku, Sapporo 060, Japan)
Fax, +81-11-717-7569, e-mail. tsuka@vetmed. hokudai. ac. jp

Abstract.   In order to solve traffic problems and to understand possible epidemic risks resulting from the feeding of wild red foxes, *Vulpes vulpes*, the acquisition of food begging behavior by foxes in the Shiretoko National Park, Hokkaido, Japan was studied.   Foxes were individually identified and their behavior was observed from June to October each year from 1992 to 1994. The locations of family territories and denning sites were established, and the degree of their tolerance to humans was investigated, and the relevancy of these factors in food begging behavior was examined. The development of food begging behavior among individuals less than one year old was strongly correlated ($p < 0.01$) with their dens being within 20 m of the road edge.   Most juveniles which were not born in dens near the roadside showed no food begging behavior and most individuals more than one year old, which had not previously shown such behavior did not acquire it at all, strongly suggesting that food begging behavior was predominantly acquired by juveniles denning near roads.   Thus, preventing foxes from denning near roads should be an effective means to obstruct the acquisition of begging behavior.

Key words : food begging behavior, tolerance to humans, Shiretoko National Park, *Vulpes vulpes schrencki*.

Feeding wildlife is considered to be an undesirable recreation, which not only has considerable impact on wildlife (Nature Conservation Society of Japan 1978), but may also lead to risks for humans.   Injuries to people have been caused for example by grizzly, *Ursus arctos*, and black bears, *U. americanus* (McCullough 1982, Robinson and Bolen 1989, Herrero and Fleck 1990, Wright 1992) in North America and by Japanese monkeys, *Macaca fuscata* (Wada 1989) in Japan, and Japanese monkeys have caused damage to crops as a result of feeding (Nature Conservation Society of Japan 1978, Wada 1989).

In the Shiretoko National Park (SNP), many red foxes, *Vulpes vulpes schrencki*, have been fed by park visitors since 1970 (Tsukada 1994, Watanabe and Tsukada 1996).   The foxes have appeared on the road through the SNP during the daytime, some of them even lying down in the center of the road in order to stop vehicles and to obtain food from the drivers and passengers.

Some traffic accidents have occurred as a result of this behavior, when drivers have dodged foxes on the road.   Traffic jams have also occurred in the SNP when vehicles have parked haphazardly to feed the foxes on the road during the peak visitor period of the summer vacation.

Some foxes in the SNP have become tame enough to be fed by hand by visitors who, by doing so, unwittingly run the risk of infection, because red foxes in Hokkaido are a definitive host of *Echinococcus multilocularis* which causes alveolar hydatid disease in humans (Yamashita 1978).   Humans become infected accidentally by ingestion of the parasite's eggs deposited in fox feces (Yamashita 1978).   Kondo *et al.* (1986) found that between 10% and 60% of the foxes in eastern Hokkaido were infected with this parasite.   Coproantigen detection of fox feces (Nonaka *et al.* 1996) has confirmed the presence of echinococcus infection among some foxes inhabiting the SNP (Nonaka in prep.).   When dogs are infected by *Echinococcus multilocularis*, various body surfaces, particularly of the anal area, the claws, femora and nose are typically contaminated with echinococcus eggs (Yamashita 1978), and this pattern is believed likely in infected foxes.   As a consequence, direct physical contact with infected foxes begging for human food may, therefore, increase the risk of the transmission of this disease.

Although prohibiting park visitors from feeding wild foxes would help resolve these problems, there is no legal foundation for such a prohibition.   In reality, it is very difficult to stop visitors to the SNP from tossing feeding foxes by hand, even where signs prohibiting the feeding of wild animals have been set up.   Controlling the food begging behavior of red foxes is the obvious alternative, however previous studies have not attempted to clarify the conditions under which foxes come to be fed by people (Aoi *et al.* 1988).

In this paper, a study analyzing how foxes come to be fed by people is described, and means for controlling fox behavior are suggested.

MATERIALS AND METHODS

1.   Study area

The field study was carried out in the Shiretoko National Park (SNP) (44° 06'N, 145° 03'E) in the eastern part of the northern Japanese island of Hokkaido. Every year 1.5 million tourists visit the SNP.   An intensive investigation was conducted along the approximately 20 km of main road which crosses the SNP. Half of the length of the road is paved and about 7.5 m wide, while the other half is narrow (5 m wide) and unpaved (Fig. 1).   The whole road is closed throughout winter, from November to May, because of deep snow.

2.   Observation of food begging behavior

The food begging behavior of foxes was defined as : 1) appearing on or alongside the road during the daytime when people might visit, and 2) staying in positions where drivers or passengers could notice them.

Forty-three foxes (18 males and 25 females) begging for food along the road
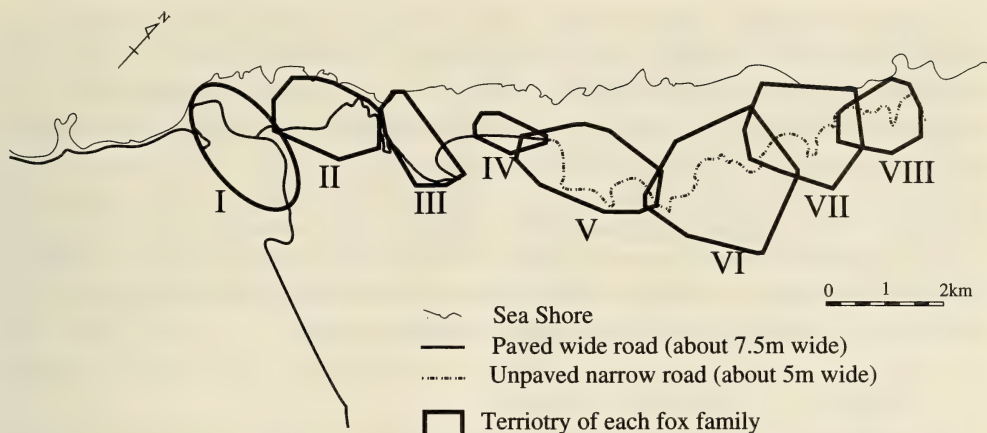
Fig. 1. Distribution of fox family territories in Shiretoko National Park. Territories II, IV, V, VI, VII and VIII are drawn on the basis of 95% Minimum Convex Polygons (MCP) of all locations of radio-collared females in reproductive condition from May to August 1993. Territory III is drawn by 95% MCP of all locations of a radio-collared male from May to August 1994. Territory I is roughly drawn from many sightings of its residents.

were captured either by using handmade blow darts, or padded foothold traps (Victor Soft Catch, Wood Stream Co.), and fitted with individually identifiable colored ear tags (Allflex 25, Allflex New Zealand Ltd.). Standard morphometrics such as body weight, body length and hind leg length were recorded. Individuals were assigned to one of three age classes (less than one year old, one year old, and more than one year old) which were determined by the annual attrition of incisors (Harris 1978). Because female foxes are capable of breeding at 10 months of age (Ables 1975), animals less than one year old were considered to be juveniles, and those one or more years old were adults. Whether pups were being reared by females was evaluated by the development of their nipples from May to July in 1992–1994.

Foxes which could not be captured but which were observed begging several times were identified by unique features such as pelage characteristics and scars, and by the location at which they appeared.

Observations of foxes begging were made from a car while driving along the main road through the SNP during the period from June to October in 1993 and 1994. Trips were conducted every two hours from 07 : 00 to 17 : 00 for two days each month, with additional trips made at random. Observations in 1992 were only conducted at random. The number of days of observation each month varied from seven to 21 (Table 2).

3. Identification of fox families

As in other areas, related adult foxes in the Shiretoko National Park usually shared common territories (Macdonald 1981, 1983, Murder 1985, Poulle *et al.* 1994, Tsukada 1997). Therefore, foxes appearing along the same sections of the SNP road were judged to share the same territories, while foxes

appearing at several widely dispersed locations were regarded as itinerants without territories.   When there was at least one female in reproductive condition among members sharing a territory, the group was defined as a "reproductive family".   The size of a reproductive family was counted in each territory during the years of the study.

Fox dens were searched for by tracking in the snow during the winters of 1992 and 1993.   As some reproductive families built their dens near the road, signs of these den sites were searched for along the "roadside", that is within 20 m of each shoulder of the road during the period from May to August when the dens are usually occupied and used for pup-rearing.

4.   The Degree of tolerance to humans

To evaluate the degree of tolerance to humans, each fox was approached and the distance at which the fox began to flee was recorded (Table 1).   The investigation was conducted more than once for each animal between June and October 1994.   Mean scores were calculated for each animal and used as an index of the degree of their tolerance towards humans.

Table 1.   The scores and criteria of degrees of tolerance to humans in foxes.

Scores	Criteria
	Fox begins to flee ;
1	when a vehicle approaches
2	when the researcher alights from the vehicle at ≥ 5 m
3	when the researcher approaches to a distance ≥ 5 m
4	when the researcher approaches to a distance ≥ 3 and < 5 m
5	when the researcher approaches to a distance ≥ 1 and < 3 m
6	when the researcher approaches to a distance ≤ 1 m or does not flee

RESULTS

Fifty foxes were observed begging for food from people during the study period.   Twenty-eight of these (12 males and 16 females) were adults, six (2 males and 4 females) were juveniles at first but later became adults and 22 (sex unknown) were juveniles.   Eight territories were confirmed by radio-tracking (Table 2), four territories (I–IV) were located along the wide paved section of the SNP road, while the other four (V–VIII) were located along the narrow unpaved section (Fig. 1).   The locations and sizes of these territories were essentially stable during the years 1992–1994.

The number of adult foxes observed begging, and the time they spent begging each year varied among the various territories.   In territories I, II and III, the maximum number of adults observed begging was two, whereas in territory VI it was three, and in IV, V, VII and VIII, it was four.   The maximum number of adults begging in each territory and in each year was significantly higher in territories along the narrow unpaved section of the SNP

road than in territories along the wide paved section (U-test, $p < 0.01$). In territories I and II, adults continued begging until August, while in territories IV–VIII, adults continued the behavior until October (Table 2). The total number of adults begging decreased during September and October each year. Some adults such as the breeding males in territories I and VI and a breeding female in territory III were not observed begging during the study period, even though other members in the same territories were (Table 2). These foxes and a breeding male in territory II in 1994 were observed to avoid all humans.

Juveniles from a total of 11 reproductive families were observed begging for food (Table 3). The reproductive families with at least one juvenile begging shared one important feature in common in the selection of their den sites,

Table 2. Identified foxes which showed food begging behavior each month from 1992 to 1994. Solid circles and triangles indicate adult and juveniles begging human food, respectively. Open circles and triangles indicate adult and juvenile foxes, which did not beg human food, respectively. Figures under months are observation days in 1992, 1993 and 1994 from left to right.

Territory	Fox code	Sex	Jun 19, 7, 22	Jul 13, 24, 26	Aug 21, 28, 20	Sep 13, 12, 24	Oct 14, 12, 23
I	Fu	F	○ ● ●	○ ● ○	○ ○ ○	○ ○ ○	○ ○ ○
II	Hi	M	● ●	● ●	● ●	○ ○	○ ○
	Ne	F	● ● ●	● ● ●	● ● ●	○ ○ ○	○ ○ ○
III	Mo	M	● ● ●	● ● ●	● ●	○ ○	○ ○
IV	No	M	● ● ●	● ● ●	● ● ●	● ● ○	● ● ●
	Ki	F	● ● ●	● ● ●	● ● ●	● ● ○	● ● ●
	Th	F	○ ● ●	○ ● ●	○ ● ●	○ ○ ○	○ ○ ○
V	Na	M	● ● ●	● ● ●	○ ● ●	○ ○ ●	● ● ○
	Oi	F	● ● ●	● ● ●	● ● ●	● ● ●	● ○
	Ie	M	●	●	●	●	●
	Tu	F	● ● ●	● ● ●	● ●	● ●	● ●
	Oeb	M	△ ●	▲ ●	▲	▲	▲
VI	Se	F	● ● ●	● ● ●	● ● ●	○ ● ●	○ ● ●
	Sks	F	▲ ●	▲ ●	▲ ●	▲	
VII	Hy	M	● ● ●	● ● ●	● ● ●	○ ● ○	● ● ○
	Ga	F	● ● ●	● ● ●	● ● ●	○ ● ●	● ● ○
	Gak	F	△ ● ●	▲ ● ●	▲ ● ●	△ ○ ○	▲ ● ●
	Gdk	F	△ ●	▲ ●	▲ ●	▲ ●	▲ ○
VIII	Ka	M	● ●	● ●	● ○	○ ○ ●	○ ● ○
	Ya	F	○ ● ●	○ ● ●	○ ● ●	○ ○ ○	● ● ○
	On	F	○ ● ○	○ ● ○	● ● ●	○ ○ ○	● ○ ○
	Or	F	○ ● ○	○ ● ○	● ● ○	○ ● ○	○ ○ ○
	Ty	F	○ ● ○	○ ● ○	● ● ○	○ ○ ○	● ○ ○
Itinerant	Si	M	○ ○ ○	○ ● ○	● ○ ○	○ ○ ●	● ○ ○
	Ma	M	○ ○ ○	○ ○ ○	● ○ ○	○ ○ ○	○ ○ ○
	M	M	○ ○ ○	● ○ ○	● ○ ○	○ ○ ○	● ○ ○

Table 3. Correlationship of begging human food by juvenile foxes with the shift of their dens toward the roadside (20 m or less from road shoulders). Begging food by one or more juveniles in each reproductive family is indicated as "+". Den shift toward the roadside of the main road in the national park is indicated as "+", and the earliest date of confirmation for the den shift is shown in parentheses.

	Reproductive family							
	I		II		III		IV	
	Begging	Roadside den	Begging	Roadside den	Begging	Roadside den	Begging	Roadside den
1992	−	−	no reproduction		−	+ (6 Aug)	−	−
1993	+	+ (17 July)	−	−	−	−	+	+ (20 July)
1994	+	+ (22 June)	−	−	−	−	+	+ (12 July)
	V		VI		VII		VIII	
	Begging	Roadside den	Begging	Roadside den	Begging	Roadside den	Begging	Roadside den
1992	+	+ (29 May)	−	−	+	+ (3 July)	−	−
1993	+	+ (9 June)	+	+ (24 June)	+	+ (23 June)	−	−
1994	+	−	−	−	+	+ (29 June)	−	−

that is, 10 out of these 11 families moved their dens to the roadside during June and July (Table 3). Conversely, 11 out of the 12 reproductive families which did not move their dens to the roadside also had no juvenile which begged (Table 3). Therefore, whether a juvenile showed food begging behavior or not was significantly correlated with whether its family moved their den to the roadside or not (Fisher's exact probability test, $p < 0.01$). However, the number of families wherein at least one juvenile begged did not differ significantly between the territories along the wide paved road and those along the narrow unpaved section (Fisher's exact probably test, $p > 0.05$).

The degree of tolerance towards humans was measured among 21 adult foxes. The mean score, 3.66 (range : 1.4–5.9, *SE* : 1.20) did not differ between age classes, sexes or the reproductive conditions of females (*U*-test, $p > 0.05$; Tables 2 and 4). The foxes in the territories along the narrow unpaved section of the SNP road, however, showed a significantly higher degree of tolerance to humans than those in the territories along the wide paved section ($p < 0.01$). The most highly tolerant foxes lay down in the center of the road in order to

Table 4. The degree of tolerance to humans among adult foxes which showed food begging behavior in 1994 comparing age, sex, reproductive condition of females and the road-type in territories.

Fox categories	*n*	Mean	*SE*	*U*-test
One year old	5	4.62	0.37	
More than 1 year old	16	3.36	0.29	$p > 0.05$
Adult male	6	3.35	0.34	
Adult female	15	3.78	0.35	$p > 0.05$
Female in reproductive condition	10	3.47	0.42	
Female in non-reproductive condition	5	4.41	0.56	$p > 0.05$
Wide paved road	7	2.54	0.38	
Narrow unpaved road	14	4.22	0.23	$p < 0.01$

stop vehicles and were willing to be fed by hand.

Only two adults began begging halfway through the study period. One of these was the male "Ka" in territory VIII, which first began begging for food in May 1993. Even on first contact, "Ka" did not flee, moreover, he approached the survey vehicle even though he had not previously taken food from visitors there. "Ka" was thereafter observed frequently even at night, and showed a high degree of tolerance with a score of 4.5. The other was the adult female "Th", which first began begging in April 1993. Her behavior was unique in that she began by fleeing as a vehicle approached, but then stayed within sight of the driver and waited to be fed. "Th" was less tolerant of humans in 1993 and this tendency did not change in 1994. Her degree of tolerance towards humans was the lowest scored (1.4) during this study.

DISCUSSION

There was a strong correlation between the acquisition of begging behavior among juveniles and denning near the road. This correlation could be accounted for partly by the fact that juveniles usually confine their activities to the area around their den until July, after which they are taken on exploratory trips by adults (Henry 1986, personal observations). None of the juveniles denning away from the roadside, however, began begging even when they were able to move around the whole of their parent's range during September and October (personal observations). This strongly supports the belief that denning near the road is an important contributory factor in the acquisition of begging behavior among juveniles. The numerous opportunities for interacting with people along the road near their den, and for contact with adults already showing begging behavior might facilitate the learning of the same habit among juveniles.

Some adults were not observed begging at any time during the study period, even though other individuals living in the same territories were. Furthermore, only two adults commenced begging during the study period. However, one of the two, the adult male "Ka" was considered to have already acquired the begging habit somewhere else before settling in territory VIII in spring 1993, because he was observed begging when he could not have had any opportunities to learn the behavior in the territory. The other individual "Th" began begging in April 1993, but differed from other foxes in that she was extremely intolerant of people. It appears, therefore, that acquiring the begging habit is difficult for adult foxes.

The degree of tolerance to humans and the duration of begging among adults differed among territories. Seasonal variation in begging behavior among red foxes in the SNP depends on the availability of its major natural food items (Tsukada and Nonaka 1996). It is assumed, therefore, that the differences in begging behavior observed among adults were related to the availability of natural foods in each territory. Indeed, each territory was located in a slightly different habitat, which would lead to differences in the

available food items among neighboring territories (Macdonald 1981).

Adults in the territories along the narrow unpaved section of the SNP road showed a high degree of tolerance to people. Two possible reasons for this should be considered. Firstly, that highly tolerant foxes choose territories along this section of the road, or join a family with such a territory. Secondly, that environmental conditions along this section of the road encourage foxes to be more tolerant.

A fox family is usually composed of a matrilineal kinship group (Macdonald 1983). Hence, migration of adult females between families does not occur. In fact, only adult males migrated into certain family territories in the study area (unpubl. data). Furthermore, the locations of the territories changed little over three years (Tsukada 1997), and had probably not changed over a longer period (Watanabe and Tsukada 1996). Therefore, the first possible reason is unacceptable. On the narrow unpaved section of the road, the view is blocked by numerous roadside trees and blind corners. Under these conditions begging foxes must endure the closer approach of vehicles and people here than on the wider paved section. Furthermore, the narrow shoulders of the unpaved section prevents foxes from taking food from visitors at a distance. Hence, foxes in territories along this section would become more tolerant than those in territories along the wider paved section. A similar effect of road structure where they usually forage on tolerance to people has also been observed among Japanese macaques (Sugiura *et al.* 1993).

In conclusion, begging is a behavior readily acquired by juvenile foxes denning near roads, but is not typically acquired by adults. Environmental factors, such as road type did not affect acquisition of begging behavior, but the degree of tolerance to people among adults did. Therefore, the most effective means of controlling begging by red foxes would appear to be to prevent them from denning near roads. This would eliminate the possibility of juvenile foxes developing the begging habit and result in diffusion of the behavior over generations. It might thus be possible to eliminate begging entirely from the study area. Because physical and human disturbance makes foxes shift their dens (Lloyd 1980, Stubbe 1980, Sargeant *et al.* 1984, Henry 1986), the selective destruction of dens near roads, and threats made to foxes denning near road by humans or dogs may both be effective means of dispersing problematic foxes. If a direct and intensive control program of foxes in the SNP is necessary, then aversive conditioning should be introduced to a limited part of their range, namely the area along the narrow unpaved road, since that is where potentially infectious (because of their likelihood of having direct physical contact with humans) foxes live.

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The age of sexual maturity in Japanese giant flying squirrels, *Petaurista leucogenys*

Takeo KAWAMICHI

Department of Biology, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan
Fax. +81-75-551-3936, e-mail. pika@sci.osaka-cu. ac. jp

Abstract. I determined the age of sexual maturity in Japanese giant flying squirrels, *Petaurista leucogenys*. The degree of testicular development was estimated in 25 males 224 times during eight years. The scrotum began to extend at the age of 7.5–8.5 months, and was slightly swollen in males 8–13 months old. Testes of 1/2–3/4 size were observed in males from 14 months onward. By the mating season when they were 21–22 months old, the proportion with full-sized testes was 57% of the males. All five males of 27–28 months of age had full-sized testes. One 22 month-old male and one 27 month-old were observed copulating. Summer-born males had slightly faster developing testes than spring-born males. None of 19 young females were observed in estrus. The attainment of sexual maturity in males at 21–22 months of age seems very late as the intermediate-sized rodents. It is suggested that mothers allow their young to remain with them for 1–1.5 years until they become sexually mature in order to increase the survival rate of their young, thereby compensating for their small litter sizes of one or two.

Key words : flying squirrels, *Petaurista leucogenys*, scrotum, sexual maturity, testis.

Rodents achieve sexual maturity at a great range of ages, depending on the species. In general, sexual maturity is reached later in larger rodents than in smaller rodent species. Beavers, *Castor canadensis*, and marmots, *Marmota monax*, for example, which exceed 5 kg in body mass, do not become sexually mature until they are two years old, whereas small voles and mice weighing less than 100 g mature very early ; *Microtus pennsylvanicus*, for example, becomes sexually mature after 25–45 days (Bourlière 1964, Eisenberg 1981). Adult Japanese giant flying squirrels, *Petaurista leucogenys*, attain weights of up to 1.3 kg (Kawamichi 1996), and are therefore presumed to become sexually mature relatively late.

 Various aspects of the ecology of the essentially nocturnal Japanese giant flying squirrel have been investigated. These include : food habits (Ando *et al*. 1985a, Kawamichi 1997) ; feeding behavior (Ando *et al*. 1984, 1985b, Funakoshi and Shiraishi 1985), and activity rhythms (Baba *et al*. 1982). No information has previously been available, however, on the age of sexual maturity in

either captive or wild populations.

The purpose of this paper, therefore, is to describe for the first time the age of sexual maturity in wild Japanese giant flying squirrels, and to discuss the factors affecting the age of sexual maturity in this species.

MATERIALS AND METHODS

The study area consisted of 0.65 km² (65 ha) of mixed deciduous and coniferous temperate forest situated adjacent to Nara City, central Japan (34° 41′N, 135°50′E ; elevation 98-150 m), (see Kawamichi 1997).   The climate of the study area is relatively mild, with snow falling occasionally in winter, but with snow-cover not lasting more than a few days.   Research into the ecology and behavior of *P. leucogenys* was conducted at this site for eight years, from April 1983 to January 1991.   A total of 977 nights were spent in the field, spread throughout each year.

I located giant flying squirrels at night, using a 9-volt searchlight, while walking at random through the study area.   All resident squirrels were identified by a combination of scars on their ears and details of their pelage with 8-16× Nikon zoom binoculars.   Very young individuals show few clearly recognizable individual characteristics, however their identification was aided by the fact that they move in close association with their mothers.

Exact dates of birth could not be determined for most individuals in the study area, so all birth dates were calculated by the addition of the mean gestation period (74 days) to the middle dates of the biannual mating seasons, those being 1 March for the spring-born litter, and 15 August for the summer-born litter.   The degree of error between calculated and real birth dates, was considered to be within one month for spring-born litters, and within two weeks for summer-born litters, because the winter mating season covered approximately two months, whereas the May–June mating season lasted one month.

When immature males were encountered, the developmental stage of their testes was assessed as belonging to one of five categories : full size, 3/4-1/2 size, at an early stage of development, the extension of a space for the scrotum, and undeveloped (Fig. 1).   After sexually maturity, the size of the testes of adult males were estimated, illustrated, and classified into four categories : full size, 2/3-3/4 size, 1/3-1/2 size, and completely regressed.   The size of the vulvae of individually identified females was also described and illustrated.

Mating behavior was observed during 16 mating seasons during the eight-year study period, and observations were made on most nights during each mating season.   Females in estrus were recognized by their swollen, pink vulvae, and by the behavior of males.   When females came into estrus, aggressive behavior among males congregating at their nests was observed.   I followed estrous females after they left their nests in order to confirm mating. Mating behavior and the identities of mating males were all recorded.

Fig. 1. Five categories of testicular development. A: Undeveloped; B: Extension of a space for the scrotum; C: Early development of testes; D: 2/3 size testes; E: Full size testes. The anus is shown under grayish pelage.

RESULTS

The Japanese giant flying squirrel has two mating seasons, the first from mid-November to mid-January, and the second from mid-May to mid-June (Kawamichi *et al*. 1987). Gestation lasts 74 days (Kawamichi unpubl.), and the addition of this period to these two mating seasons each year indicates that the two birth seasons occur mainly from early February to early April, and then from late July to late August.

Litters of one or two altricial young are born in tree cavities. They begin to appear at their nest entrance approximately 45 days after birth, and leave their nests when 59 or more days old (Kawamichi unpubl.). The individuals examined for this study were those which were observed for six or more months after first appearing outside their nests. These included 25 males (born from 17 mothers) and 19 females (from 12 mothers), which were observed for between six months and 5.5 years.

1. Development of testes

For 25 different males aged 2–28 months, the degree of external testicular development was estimated, and the size of the scrotum was recorded repeatedly, a total of 224 times. Testis condition of a total of 93 males was determined bimonthly except for males aged 2–8 months (see Fig. 2).

The first indication of sexual development in males was the extension of a very narrow space for the scrotum between 6.5–7 months of age. The scrotum began to extend at the age of 7.5–8.5 months (Fig. 1), and was slightly swollen in males 8–13 months old. A male of this age, which met an accidental death, had testes of about 1 cm in diameter. From the age of nine months, small rounded testes, in the early stages of development, were visible in the scrotum. Testes of 1/2–3/4 size were observed in males from 14 months onward, and the proportion of individuals with testes of this size increased steadily until 18 months of age (Fig. 2).
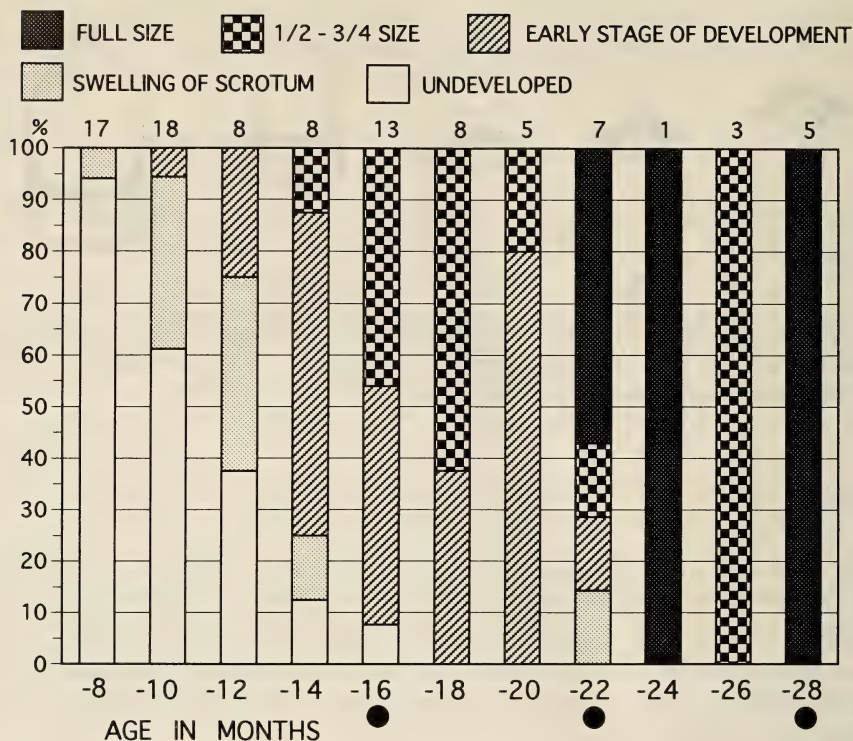
Fig. 2. Age-related development of testes. Bimonthly changes in the proportion of males with testes of various sizes. Upper figures refer to the number of males. Solid circles indicate the mating season.

Regardless of whether they were spring- or summer-born, the first opportunity to participate in a mating season came when males were 15–16 months old. At this stage, young males were fairly evenly divided between those with testes in early stages of development, or between 1/2 and 3/4 size (Fig. 2). By their second mating season, they were 21–22 months old, and the proportion with full-sized testes was 57.1%. By their third mating season, all five identifiable males of 27–28 months of age had full-sized testes.

Males which were between 15 and 17 months old were not observed mating during their first mating season, and only one 22 month-old male during its second mating season, and one 27 month-old during its third mating season were observed copulating. It is assumed, therefore, that males may become sexually mature from the age of 21–22 months.

Summer-born males had slightly faster developing testes than spring-born males (Table 1). By 9–10 months of age, spring-born males still had undeveloped testes, whereas more than half of the summer-born males of the same age had already developed a space for the scrotum ; the difference in the proportion of males with undeveloped testes was significant (Fisher's exact probability test, $p = 0.02$). During the first mating season, the difference in the degree of

Table 1.   The difference in testis development between spring-born and summer-born males. Figures represent the number of males.   Capital letters are the initials of months; bimonthly periods begin from 1st of each month for spring-born males and from 15th for summer-born males.

	Age in months										
	0-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28
Spring-born males											
	M-O	N-D	J-F	M-A	M-J	J-A	S-O	N-D	J-F	M-A	M-J
Full size	0	0	0	0	0	0	0	2	0	0	2
1/2-3/4 size	0	0	0	0	0	1	0	0	0	1	0
Early stage	0	0	0	2	4	1	1	1	0	0	0
Swelling scrotum	0	0	1	1	0	0	0	0	0	0	0
Undeveloped	6	7	3	1	1	0	0	0	0	0	0
Summer-born males											
	A-A	M-J	J-A	S-O	N-D	J-F	M-A	M-J	J-A	S-O	N-D
Full size	0	0	0	0	0	0	0	2	1	0	3
1/2-3/4 size	0	0	0	1	6	4	1	1	0	2	0
Early stage	0	1	2	3	2	2	3	0	0	0	0
Swelling scrotum	1	6	2	0	0	0	0	1	0	0	0
Undeveloped	10	4	0	0	0	0	0	0	0	0	0
Overal	17	18	8	8	13	8	5	7	1	3	5

development was also significant (Fisher's test, $p=0.05$; using data from 15-16 month old spring-born males, and from 15-18 month old summer-born males).

The rate of development of testes varied individually.   The testes of three of the 25 males observed developed very slowly; one retained a narrow space for the scrotum for 15 months, and two still had small testes when 18 and 22 months old, respectively.

Adult male Japanese giant flying squirrels experience regular regression and recrudescence of their testes, with regression occurring annually during the non-mating season in July and August (Kawamichi unpubl.).   The period of testicular regression occurs first for summer-born males when 11-12 months old ($n=4$), and for spring-born males when they are 17-18 months old ($n=2$).   The testes of all six males were, however, continuously developing.   During the second period of regression, one summer-born male 23-24 months old retained full-sized testes, while the testes of one 32 month old spring-born male regressed and redeveloped during summer.

The proportion of adult males with full-sized testes decreased during the second half of February and the first half of March (Kawamichi unpubl.).   A similar decrease in testis size was also found in three out of four 19-20 month old summer-born males (see the increased proportion of small testes in Fig. 2). The testes of one 19 month old male, however, regressed from March through the May-June mating season.

2. Sexual maturity in females

Among immature females, the size of the external vulvae increased very

slowly until their first estrus. None of 19 young females were observed in estrus during the mating season. The vulvae of five, out of the 19 females observed, were examined closely during the mating season when they were 9–10 months old. Only one of the five had slightly swollen vulvae. Another female had a similarly swollen vulva when it was 16 months old.

Young females usually dispersed from their natal territories before their first estrus (Kawamichi unpubl.), thus it was difficult into observe the age of sexual maturity. Furthermore, because of the short period of estrus, the occurrence of estrus during a particular mating season was very difficult to recognize. The data indicate, however, that young females do not come into estrus during the mating season that takes place when they are 9–10 months old.

DISCUSSION

It appears that there are three possible factors affecting the age of sexual maturity in Japanese giant flying squirrels that should be considered. The first factor is the interval between the two annual mating seasons ; the second factor is the slightly different age of sexual maturity between spring- and summer-born males ; and the third factor is social.

In seasonally breeding mammals, the timing of sexual maturity is related to the interval between mating seasons. In species such as the Siberian chipmunk, *Tamias sibiricus*, which has one short mating season each year (Kawamichi and Kawamichi 1993), mating occurs at the age of 11 months, despite their small body size. In Japanese giant flying squirrels, which have two mating seasons each year, the first mating season occurs when they are 3.5 months old, and later every six months (9.5, 15.5, 21.5, and 27.5 months). Therefore, the interval between mating seasons would, at most, postpone their sexual maturity six months.

Summer-born males reach sexual maturity slightly sooner than spring-born males. One possible reason for this is that food availability and the nutritional value of available food differ for summer- and spring-born males. Young spring-born litters begin foraging from late April onward, when their diet consists largely of leaves and buds, whereas summer-born litters begin foraging from mid-October onward when their diet consists largely of seeds (Kawamichi unpubl., Kawamichi 1997). Further study is required to establish whether this dietary difference influences the growth rate of young squirrels after weaning and therefore influences the age of sexual maturity.

Given that sexual maturity in extra-large rodents, such as beavers and marmots, occurs at two years of age (Bourlière 1956, Eisenberg 1981), the attainment of sexual maturity at 21–22 months of age in Japanese giant flying squirrels seems very late given its intermediate size. Their relatively late maturation should be considered, however, from the perspective of reproductive success. Japanese giant flying squirrels have small litters of just one or two young (Kawamichi 1996), thus the maximum number of young they can raise each year is four. Young squirrels of both sexes remain in their natal

territories for 1-1.5 years, until they are sexually mature (Kawamichi unpubl.). These facts suggest that mothers allow their young to remain with them until they become sexually mature in order to increase the survival rate of their young, thereby compensating for their small litter sizes. Furthermore, young squirrels may delay reaching sexual maturity so as to have longer to grow up in their mothers' territories.

The age of reaching sexual maturity in Japanese giant flying squirrels is likely to be determined by three inter-related factors, the growth rate of the young, their longevity, and life time reproductive success.

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Structure of a breeding nest of the Daurian pika, *Ochotona daurica*, in Mongolia

Takeo KAWAMICHI[1] and Samdannyamin DAWANYAM[2]

[1] *Department of Biology, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan*
Fax. +81-75-551-3936, e-mail. pika@sci. osaka-cu. ac. jp
[2] *Rodent Research Section, Mongolian Plant Protection Institute, Ulanbator, Mongolia*

Abstract.   We excavated a breeding nest of the Daurian pika, *Ochotona daurica*, in central Mongolia.   Four young were captured within the burrows. Three food storage chambers contained plant fragments and a large amount of fecal matter, indicating that hoarded food had been consumed during the last winter.   The nest chamber was spherical and measured 22×18×21 cm.   Most of the nest chamber was filled with piles of grasses, and these piles were presumably their resting site.   The burrow system had three entrances, and the nest chamber was connected to three burrows. Multiple nest entrances were provided ready access to refuge for pikas active on the ground surface from aerial and terrestrial predators, while multiple burrows also provide refuge against the intrusion of predators such as stoats into nest chambers.

Key words : Daurian pika, food storage, nest burrows, nests, *Ochotona daurica*.

The nest, or burrow system, has been described for four species of pikas in the genus *Ochotona* : *O. daurica* (Dmitriyev 1991), *O. rufescens* (Puget 1971), *O. pallasi* (Simirnov 1974), and *O. pusilla* (Simirnov 1974).   Although Dmitriyev (1991) revealed the distribution of nest chambers in the complicated burrow systems of a colony of *O. daurica*, for none of these four species, have the detailed structure of nest chambers, or breeding nests, been described.

The Daurian pika, *O. daurica*, occurs commonly throughout grasslands or steppes in the south-eastern corner of west Siberia in Russia, the northern half of Mongolia, and northern China (Ognev 1940).   Despite their extensive range, little information on their natural history has been gathered, other than details of their reproduction, vocalization, and of the hay piles accumulated at their nest entrances (Loukashkin 1940, Zevegmid 1975, Orr 1977, Dlamtcheren *et al*. 1989, Dmitriyev 1991).

The purpose of this paper, therefore, is to provide detailed information on the structure of a breeding nest of the Daurian pika, and to discuss the function of its complicated burrow system in steppe habitats.

MATERIALS AND METHODS

The study area was in the grassland at Baan-tsagaan Som (Village) (45°50′
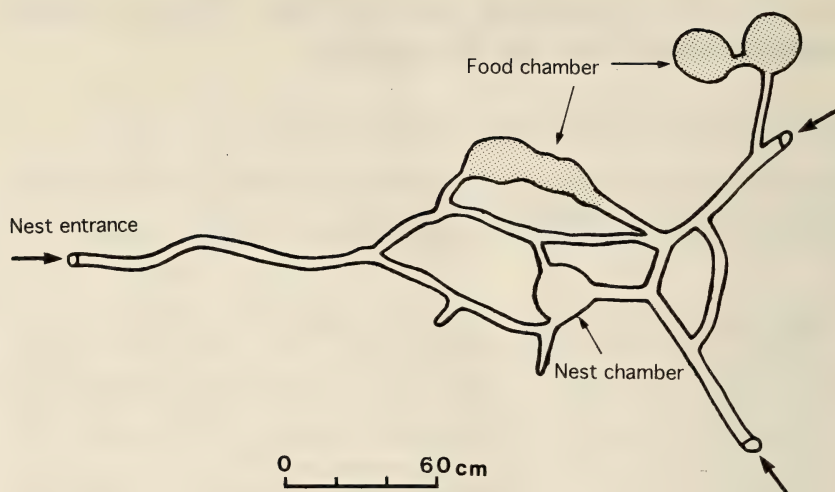
Fig. 1   Horizontal section of burrow system of *Ochotona daurica* consisting of one nest chamber, three vacant food chambers (hatched), and three nest entrances (large arrows).

N, 99°30′E), 126 km south-west of Bayan-hongor, Bayan-hongor Prefecture, central Mongolia.

We located one breeding nest of *O. daurica* after observing that young pikas repeatedly entered and left a burrow entrance on 14 July 1992.   The burrow system was excavated carefully with a shovel, a knife, and by hands, and measured to the nearest centimeter. Three-dimensional measurements are given as : length×width×height.

RESULTS

1. Burrow system

The burrow system had three entrances to the ground surface (Fig. 1). These entrances, measuring 5 cm in diameter, gave access to sloping burrows which were 5-7 cm in diameter.   In vertical section, the burrows were round with flat bottoms, and extended to a depth of 20-38 cm.   The distance from the left entrance to the two entrances on the right (Fig. 1) was 280 or 290 cm.

Three food storage chambers were detected.   Two spherical chambers measured 21×21×18 cm and 22×22×20 cm, respectively.   The ceilings of the two chambers were 20 and 21 cm below ground.   The remaining chamber was 55 cm long and 17 cm wide at its widest point.

The three food storage chambers contained small quantities of plant fragments and a large amount of fecal matter which was not old, indicating that food had been hoarded and consumed during the winter of 1991-1992. Feces were only noticeable in these three food storage chambers and the corner of the nest chamber.
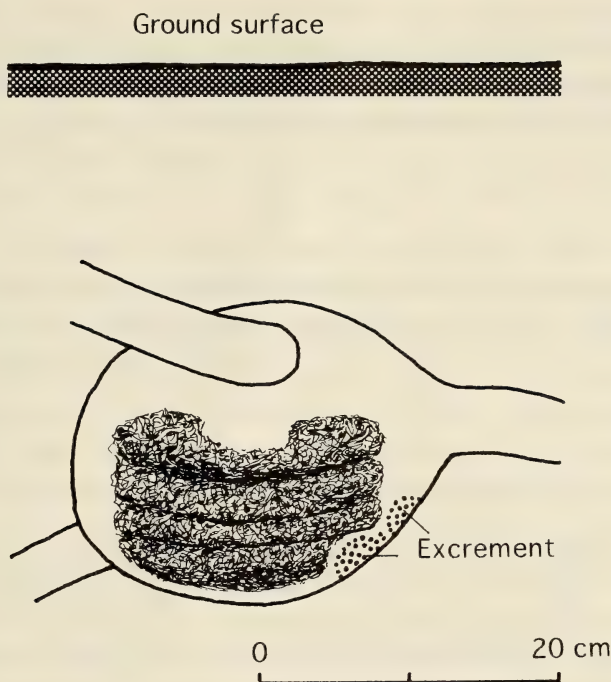
Ground surface

Fig. 2.   The structure of a nest chamber.   The nest chamber was linked to the surface by three burrows ; 4–5 layers of dry grasses were accumulated, and two mounds of feces were at the bottom.

Prior to our excavation, an adult pika, presumably the mother, ran away from the burrow system.   We were able to capture four young of similar sizes within the burrows (body weight 34.0 ± 0.9 (*SE*) g ; ear length 12.3 ± 0.4 mm ; hind foot length 22.5 ± 0.5 mm ; total length 106.5 ± 2.3 mm, $n=4$).   These observations indicated that this nest was being used for rearing young, and because of the large amount of feces found in the three chambers, we believe that this burrow system was in continuous use throughout the winter of 1991-1992 and the spring of 1992.

2. Nest chamber

The nest chamber was spherical and measured $22\times18\times21$ cm.   The ceiling of the nest chamber was 16 cm below ground.   The nest chamber was connected to three burrows each running in a different direction (Fig. 1).   There were two mounds of feces in the corner of the nest chamber.

Most of the nest chamber was filled with fibrous grasses as nesting material.   These grasses, presumably the same as their food plants, included both leaves and roots.   Most of these grasses were curled and intertwined, so that the piles formed a soft cushion.   Piles of grasses ($18\times21\times13$ cm) were composed of four or five layers (Fig. 2).   The top layer consisted of a 4 cm thick dried disk weighing 42 g.   The central part was depressed, and was

presumably their resting site. The distance from the top layer to the chamber's ceiling was 8 cm. Lower layers were less dry, and indicated that *O. daurica* had repeatedly added fresh piles of nesting material on top of material which had lost its softness and/or become damp.

DISCUSSION

Ochotona pikas exhibit three types of habitat preference. They either occupy rocks, steppes, or habitats intermediate between these two (Kawamichi 1971, Smith 1988). Of the four species whose nests or burrow systems have been described, *O. daurica* (Dmitriyev 1991) and *O. pusilla* (Simirnov 1974) are "steppe dwellers", and *O. rufescens* (Puget 1971) and *O. pallasi* (Simirnov 1974) are intermediate types. All four species have complex burrow systems with many entrances. Rock dwelling species inhabit rock slides, however their nests have not so far been described, because of the difficulty of excavating rock slides.

Dmitriyev (1991) described the distribution of burrows in a colony of *O. daurica*. The largest burrow system had three nest chambers and 42 nest entrances within an area of 3.8×2.8 m (calculated from Dmitriyev's [1991] Fig. 1). In both Zevegmid (1975) and Dmitriyev's (1991) colonies, burrows were 5 cm in diameter, whereas they were 5-7 cm in this study. The diameter of the nest chambers was 27.6 ± 2.5 (*SE*) cm (range$=22$-36, $n=5$, calculated from Dmitriyev's [1991] Fig. 1), which was similar to the 22 cm of this study, although Zevegmid (1975) found them to be much smaller at 11-12 cm. Dmitriyev's (1991) burrow system, extending 22-30 cm below the surface, was very similar in depth to ours (20-21 cm ; this study), whereas Zevegmid's (1975) burrow system was, at 11-12 cm, much shallower.

O. daurica typically accumulates large amounts of hay at its nest entrances for winter food (Loukashkin 1940, Ognev 1940, Zevegmid 1975, Orr 1977, Dlamtcheren *et al.* 1989). By mid-July, however, when we excavated the nest, there were no signs of plant material accumulations around the nest entrances. We were, however, able to describe, for the first time, the existence of food chambers underground in this species, though this is by no means unique to the genus, as Puget (1971) has described a similar burrow structure with food chambers underground and accumulated hay piles at nest entrances for *O. rufescens*. Although the food storing capacity of *O. daurica's* chambers does not seem to be great enough for the length of the winter in this region, the large amount of feces in the chambers suggests that they carried hay from the nest entrances into these chambers where they fed on it.

It is considered that the complex burrow system serves important functions. Pikas are often active above ground, thus having many nest entrances provided ready access to refuge from predators such as snowy owls, *Nyctea scandiaca*, corsac foxes, *Vulpes corsac*, wolves, *Canis lupus*, and particularly upland buzzards, *Buteo hemilasius* (Ognev 1940). Conversely, pikas underground are able to flee to the surface, escaping from ground predators such as

the stoat, *Mustela erminea*, which penetrates their burrow systems, by using one of the many burrows.   Dmitriyev (1991) found each of six nest chambers to be connected to the surface by 2–3 burrows, as did we, and Simirnov (1974) found that *O. pallasi* chambers were similarly connected to the surface by three burrows and *O. pusilla* chambers by five burrows.   These facts indicate that multiple burrows also provide refuge against the intrusion of predators into nest chambers.

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Behavioural and reproductive ecology of the dog-faced fruit bats, *Cynopterus brachyotis* and *C. horsfieldi*, in a Malaysian rainforest

Kimitake FUNAKOSHI[1] and ZUBAID Akbar[2]

[1]*Biological Laboratory, Kagoshima Keizai University, Kagoshima 891-01 Japan*
Fax. +81-99-261-3299, e-mail. funakoshi@kkis. ac. jp
[2]Jabatan Zoologi, Fakulti Sains Hayat, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract.   Roosting, foraging and reproductive aspects of two species of dog-faced fruit bats, *Cynopterus brachyotis* and *C. horsfieldi*, were examined in Ulu Gombak, Selangor, West Malaysia. The day roosts of *C. horsfieldi* were sparsely distributed and were found mainly in palms, whereas roosts of *C. brachyotis* were abundant, and mostly found in non-palm tree species. Males of both species frequently changed their roosts. The nocturnal activity patterns of the two species were different. The initial peak of flight activity of *C. brachyotis* was two hours sooner after sunset than that of *C. horsfieldi*, and its flight activity gradually declined during the night. In *C. horsfieldi* flight activity decreased around midnight then increased again three hours before sunrise. The home ranges of *C. horsfieldi* were larger than those of *C. brachyotis*, however these were non-exclusive ranges with individual home ranges of both species overlapping extensively. In association with their greater home range size, *C. horsfieldi* also tended to move further than *C. brachyotis* (when comparing means of greatest distances moved). The diets of the two species also differed, with *C. brachyotis* eating fruits, flowers and leaves, whereas *C. horsfieldi* ate *Ficus* fruits virtually throughout the year. The wet weight of figs carried by *C. brachyotis* per feeding bout averaged 7.9 g, while *C. horsfieldi* carried on average 17.8 g of figs. The average distances between feeding roost sites and fruiting *Ficus variegata* trees were 50-78 m. *Cynopterus brachyotis* probably produces two or three litters per year whereas *C. horsfieldi* has two litters.

Key words : *Cynopterus*, foraging behavior, home range, nocturnal activity, reproductive cycle.

A wide range of frugivorous bats occur abundantly in tropical rainforests. These belong to the Pteropodidae in the Old World, and the Phyllostomidae in the New World tropics.   In Old World tropical forest habitats, many fruit bats are to be found in tree foliage or in tree hollows (Lim 1966, Lekagul and McNeely 1977, Medway 1983, Payne *et al*. 1985).   Relatively few papers concerning the comparative ecology of sympatric pteropodids have been published

(but see Jones 1972, Wolton *et al*. 1982, Marshall and McWilliam 1982, Heideman and Heaney 1989, Kitchener *et al*. 1990).   In Peninsular Malaysia, two species of dog-faced fruit bats, *Cynopterus brachyotis* and *C. horsfieldi*, are common and often occur sympatrically (Lim 1966, Lekagul and McNeely 1977, Heller and Volleth 1989).   Although it is known that both species feed almost exclusively on plants, taking floral resources and fruit (Lim 1970, Boon and Corlett 1989, Kitchener *et al*. 1990), relatively little is known, however, about the nocturnal activity and feeding behavior of *Cynopterus* species (Boon and Corlett 1989, Heller and Volleth 1989, Bhat 1994), and no publications have addressed the details of their home ranges.   Furthermore, only a few papers cover aspects of the breeding cycles of these two *Cynopterus* species (Lim 1970, 1973, Medway 1983).   Our aim here, therefore, is to present comparative information on a range of aspects of the life styles of *C. brachyotis* and *C. horsfieldi*, in particular, details of their roosting sites, nocturnal activity, food habits, foraging behavior, home ranges and reproductive cycles.

MATERIALS AND METHODS

This study was conducted at the Field Studies Center of the University of Malaya, in a 125 ha reserve of partially disturbed rainforest in Ulu Gombak, Selangor, West Malaysia (3°20′N, 101°45′E, elevation *ca*. 200 m).   The total annual rainfall at Ulu Gombak is about 2,500 mm per year, with a major peak in October and November.   There is very little seasonal variation in temperature with a mean monthly low of 23°C and a mean monthly high of 26°C (Marshall 1970, Medway 1972).   The vegetation of the area consists of secondary forest in which several species of bamboos, palms, dipterocarps, figs and peppers are abundant but patchily distributed, with a bushy area on the western side and open forest on the east.

Mist netting with 12 m×2 m nets was conducted for 4–8 nights each month from August 1992 to March 1994.   Mist nets were set at 8–10 locations within about 10 ha and were checked at hourly intervals from sunset to sunrise.   The time of capture, location, age, forearm length, weight, sex and reproductive condition of each bat caught, were recorded, and each was marked individually with a flanged wing-band (Lambournes Ltd) before release.   The two species were easily distinguished because *C. horsfieldi* is larger, and has squarer cheek teeth with extra cusps, than *C. brachyotis* (Payne *et al*. 1985).   Females in late stages of pregnancy were identified by their weights being greater than 35 g in *C. brachyotis* and greater than 65 g in *C. horsfieldi* ;   the presence of a fetus could be detected by abdominal palpation.   Lactating females could be recognized by the swollen skin around their nipples, and by the fact that milk could be expressed.   Young individuals were easily identified because their body mass were less than 25 g (*C. brachyotis*) or less than 45 g (*C. horsfieldi*), and because their finger joints were less knobby and more evenly tapered than those of adults.   Wing bands were fitted carefully so that they were free to slide over the wing membranes without causing damage or injury.

The distribution of *Ficus* trees and feeding sites were mapped. Pellets of regurgitated material were collected beneath feeding roosts, and fecal and regurgitated pellet samples were collected after holding captured bats in cloth bags. Ten *Cynopterus brachyotis* and eight *C. horsfieldi* were fitted with radio transmitters (Alkitec Co. Ltd. Model TLM-6). These packages, weighing about 2 g (less than 6.5% of body weight), were glued to the fur on their backs. Radio telemetry of these individuals, between 28 July and 12 August 1993, allowed us to track them to their roosts, the locations of which were determined every day by triangulation, using a hand-held Yagi antenna (Alkitec Co. Ltd. Model CM-6H) and a portable receiver (Yaesu Radio Co. Ltd. Model FT-690 mkII). The location of fruit trees or feeding sites being used by the radio-tagged bats at night was confirmed by on site inspection the following day. Their home range sizes were calculated subsequently using the minimum convex polygon method (Mohr 1947).

RESULTS

1. Relative abundance and body size

Although nine species of fruit bats were recorded from the study area, *C. brachyotis* and *C. horsfieldi* contributed the overwhelming majority of records. Of the 754 individual fruit bats captured, 502 (66.6%) were *C. brachyotis* and 155 (20.6%) were *C. horsfieldi*. Both species were present in almost every month
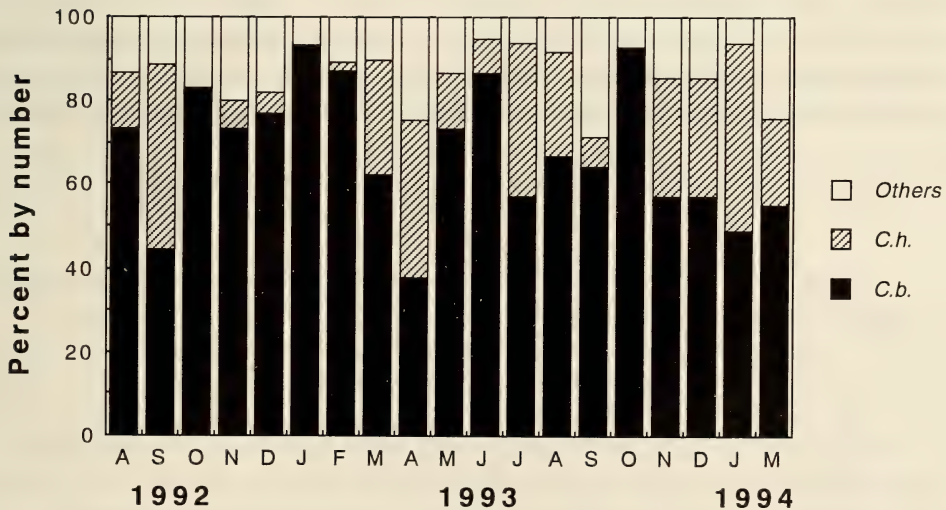
Fig. 1. Seasonal changes in the composition of fruit bats captured by mist net. Abbreviations: *C. b.* = *Cynopterus brachyotis*; *C. h.* = *C. horsfieldi*.

(Fig. 1). The other seven species, *Rousettus amplexicaudatus*, *Macroglossus sobrinus*, *Balionycteris maculata*, *Eonycteris spelaea*, *Megaerops ecaudatus*, *Chironax melanocephalus*, and *Penthetor lucasii*, were far less abundant and were only captured occasionally in the study area.

Adult *C. horsfieldi* were found to be both significantly larger (*t*-test, $p <$ 0.001 applied to forearm length), and significantly heavier (*t*-test, $p < 0.01$) than adult *C. brachyotis*, and females of both species averaged larger and heavier than their respective males.   Adult male *C. horsfieldi* had forearms measuring 74.3±3.1 (*SD*) mm, and they weighed 56.7±6.1 (*SD*) g (*n*=19), while adult females measured 75.1±2.3 mm and weighed 59.7±6.9 g (*n*=26).   Adult male *C. brachyotis* measured 60.5±2.4 mm, and weighed 30.6±3.8 g (*n*=23), whereas adult females measured 61.4±2.5 mm and weighed 33.1±4.0 g (*n*=40).

2. Day roosts

The daytime roosts of *Cynopterus horsfieldi* were found mainly in the eastern portion, or along the periphery, of the study area (Fig. 2).   They roosted in trees, preferring the axilla of palm fronds of trees such as *Cocos nucifera* and *Corypha* sp.   There were fewer than 20 palm trees over five metres tall in the study area.   Two radio-tagged male *C. horsfieldi* changed roosts every 1–7 days, while four radio-tagged females changed roosts less often (every 3–14 days).

The day roosts of *C. brachyotis* were mostly in dense foliage more than five metres above ground either in trees, such as *Durio zibethinus* (Bombacaceae) or in bamboos such as *Gigantochloa scortechenii* (Bambusoideae).   Roosts were abundant, widely scattered in the study area with many being difficult to locate precisely due to the dense foliage, and they mostly occurred in trees other than palms (Fig. 2).   Three radio-tagged male *C. brachyotis* made only transient use of foliage roost sites, occupying each site for only 1–5 days before moving on to another site.   One of the males changed its roost almost everyday (Fig. 3).

Fig. 2.   Location of day roosts of *Cynopterus brachyotis* (▼) and *C. horsfieldi* (▽) tracked in July-August 1993.   P=palm trees where bats roosted.
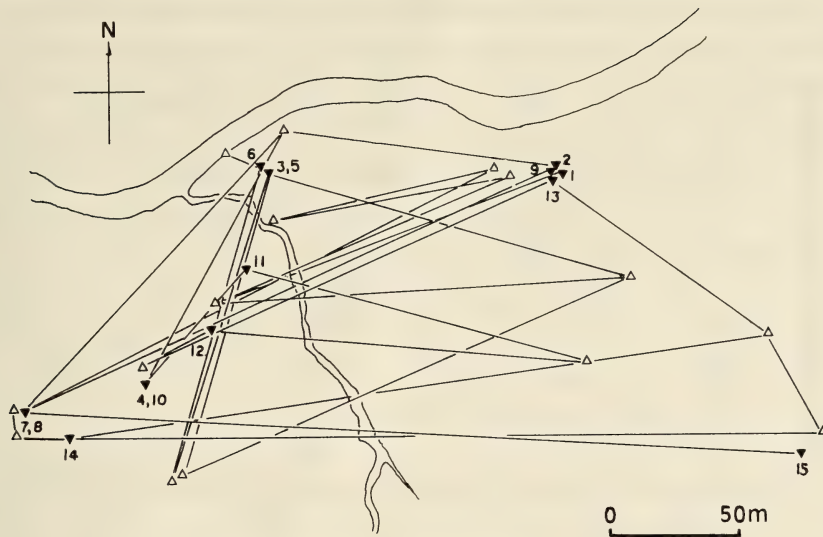
Fig. 3. Flight movements of an adult male *Cynopterus brachyotis* between 28 July and 12 August 1993. Locations of day roosts are indicated by solid triangles and those of feeding sites or resting rooosts by open triangles. Figures show the successive days on and after 28 July 1993.

In contrast, four radio-tagged female *C. brachyotis* changed roosts only every 2 –13 days, with most only occasionally changing their roosts.

3. Nocturnal activity patterns

Activity patterns were ascertained from the numbers of captures made during the night. *Cynopterus brachyotis* were most active within an hour after sunset (Fig. 4), with activity declining somewhat as the night progressed, whereas *C. horsfieldi* were most active from two to four hours after sunset, and again three hours before sunrise (Fig. 4).

4. Food habits and feeding sites

The dominant fig species in the study area was *Ficus variegata* (Moraceae), with a mean density of 1.5 large trees (about 30 m tall) per hectare. These trees fruited asynchronously, and the ripe fruits were produced on a recurrent cycle of five to eight months, the average being seven months ($n=6$). *Piper aduncum* (Piperaceae) trees were also common, occurring on the edge of the forest along the river or the road, and fruited throughout most of the year.

Figs featured heavily in the diet of *C. horsfieldi* in the Ulu Gombak study area throughout the year, with 88% of 32 feces containing fig seeds in July-August 1993. *Piper aduncum* seeds were never found in *C. horsfieldi* feces, perhaps because, owing to their weight, it was difficult for these bats to hang from the thin fruit-bearing branches of *P. aduncum*. The bulk of the diet of *C.*
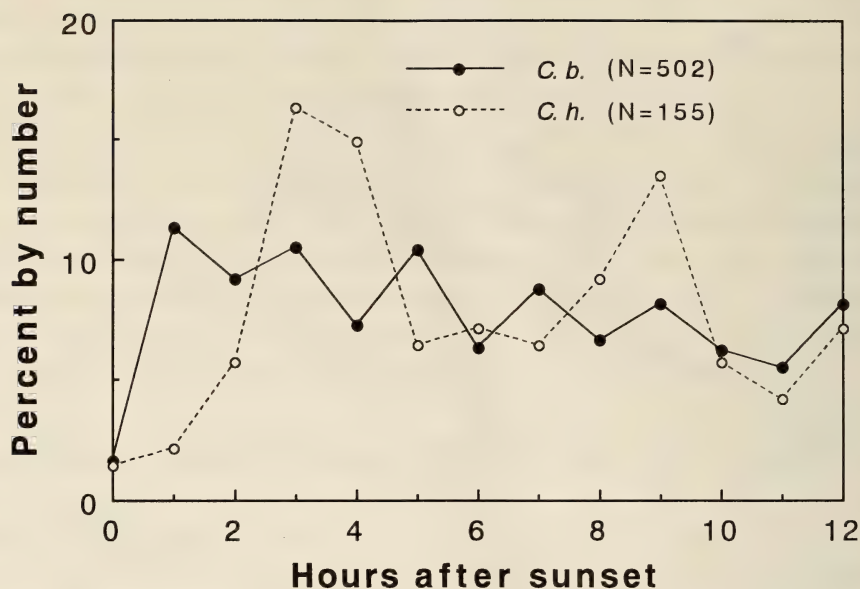
Fig. 4.   Temporal activity patterns of *Cynopterus brachyotis* (*C. b.*) and *C. horsfieldi* (*C. h.*). The graph is based on the percentage of the total number of bats captured at hourly intervals from 1992–1994.

brachyotis consisted largely of the soft fruits of *Ficus variegata*, *F. viridicarpa* and *Piper aduncum*, and the flowers of *Durio zibethinus* (Bombacaceae).   Leaf pellets were also found occasionally under its feeding sites, however the species could not be identified.   Small fig and *P. aduncum* seeds were frequently found in *C. brachyotis* feces, with fig seeds comprising 71%, and *P. aduncum* seeds comprising 25% (by number of identifiable seeds) of 48 feces in July-August 1993.

   Neither species of bat ate fruits in fruiting trees.   Instead they carried them from the foraging site to a feeding site in a neighboring tree.   Such feeding sites were located by direct observation and radio tracking, or indirectly by searching for pellets regurgitated by the bats and which fell beneath the feeding site.   The wet weight of the figs carried (into mist nets) by *C. horsfieldi* on their way to feeding sites were significantly heavier than those carried by *C. brachyotis* (Mann-Whitney *U*-test, $U=4$, $p< 0.01$).   Those carried by *C. horsfieldi* averaged 17.8 ± 5.7 (*SD*) g ($n=9$), while those carried by *C. brachyotis* averaged 7.9 ± 2.5 g ($n=11$).   When a fig was too heavy to be carried, the bats bit off pieces and carried them in their mouths.

   Although there was no significant difference between the maximum diameter of disc-shaped pellets regurgitated by the two species (Mann-Whitney *U*-test, $Z=-1.76$, $p<0.05$), fresh pellets from *C. horsfieldi* were significantly heavier than those from *C. brachyotis* (Mann-Whitney *U*-test, $Z=-3.73$, $p< 0.001$).   Fresh pellets produced by *C. horsfieldi* averaged 17.0 ± 1.3 (*SD*) mm in

maximum diameter and weighed 123.3 ± 25.3 mg dry weight ($n=15$), whereas those of *C. brachyotis* averaged 15.9 ± 1.3 mm and 94.4 ± 19.0 mg ($n=41$).

The distances between *Ficus variegata* trees bearing ripe fruits and neighboring feeding sites averaged 59.4 ± 14.1 (*SD*) m ($n=21$) and 78.3 ± 21.8 m ($n=12$) in July and August 1993, and 50.4 ± 21.7 m ($n=11$) in March 1994 (Fig. 5). The shortest distances between *F. variegata* trees averaged 35.1 ± 14.6 (*SD*) m ($n=9$). The height of the branches used by bats as feeding sites averaged 3.3 ± 1.0 (*SD*) m ($n=14$) above the ground.
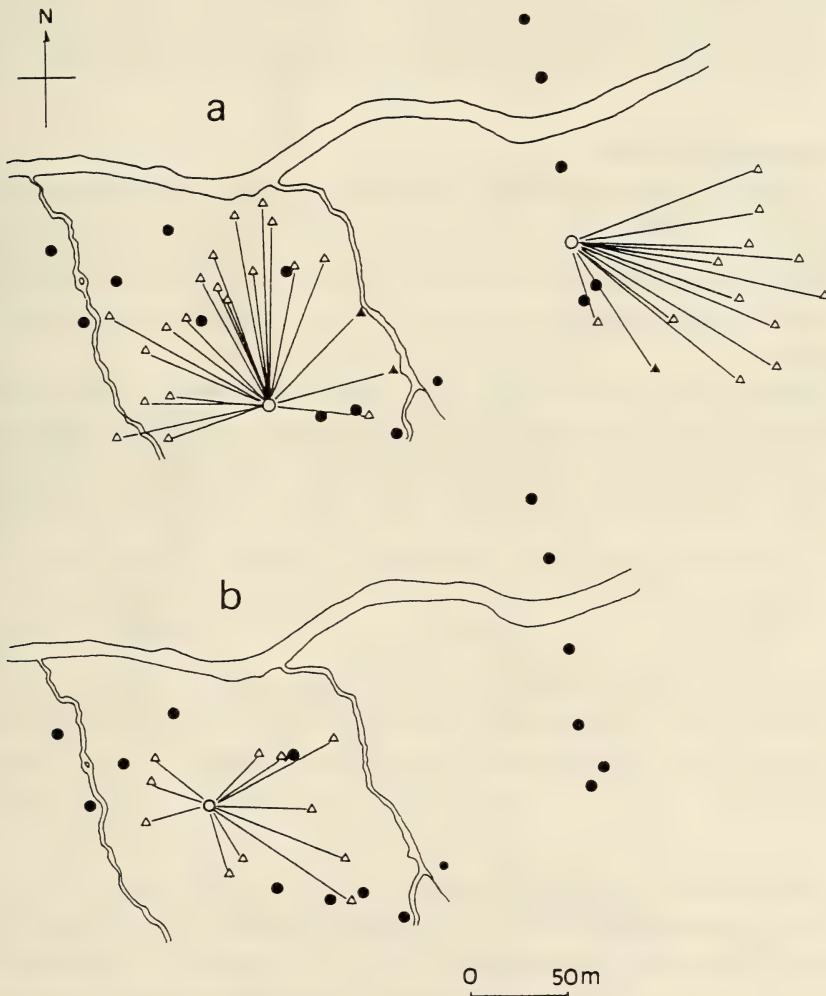
Fig. 5. Location of feeding sites of *Cynopterus brachyotis* (open triangles) and *C. horsfieldi* (solid triangles) during (a) July and August 1993 and (b) March 1994. The locations of *Ficus variegate* trees bearing ripe fruits, and trees not bearing ripe fruits are indicated by open circles and solid circles, respectively.

5. Home ranges

The distances travelled by *Cynopterus horsfieldi* were significantly further than those travelled by *C. brachyotis* (Mann-Whitney *U*-test, $U=1$, $p<0.01$). These measurements were based on the means of the greatest distances travelled by each radio-tracked individual over 12 days, which were 475 ± 105 (*SD*) m ($n=6$) for *C. horsfieldi*, and 295 ± 55 m ($n=7$) for *C. brachyotis*. The overlap of home ranges, both within and between species, was high (Fig. 6), however the home ranges of both male and female *C. horsfieldi* were significantly larger than those of *C. brachyotis* (Mann-Whitney *U*-test, $U=2$, $p<0.01$). Home ranges of adult male *C. horsfieldi* averaged 8.0 ha ($n=2$) while those of adult females averaged 5.8 ± 2.5 ha ($n=4$), whereas in *C. brachyotis*, adult male home ranges averaged 3.1 ha ($n=3$) and adult female ones averaged 3.2 ± 1.4 ha ($n=4$).

6. Reproductive cycles

Female *Cynopterus horsfieldi* in the later stages of pregnancy, and lactating females and young, were captured intermittently throughout the year, with percentages fluctuating aseasonally (Fig. 7). Although the main pregnancy peaks apparently occurred in four to six month cycles, the cycle of the occurrence of lactating females could not be clearly identified because of our small sample size.

Female *C. brachyotis* in the later stages of pregnancy, and young, were captured almost every month with the percentage fluctuating aseasonally (Fig. 7). Lactating females, however, were captured only intermittently, with lactation peaks apparently occurring in three to four month cycles. Female A005
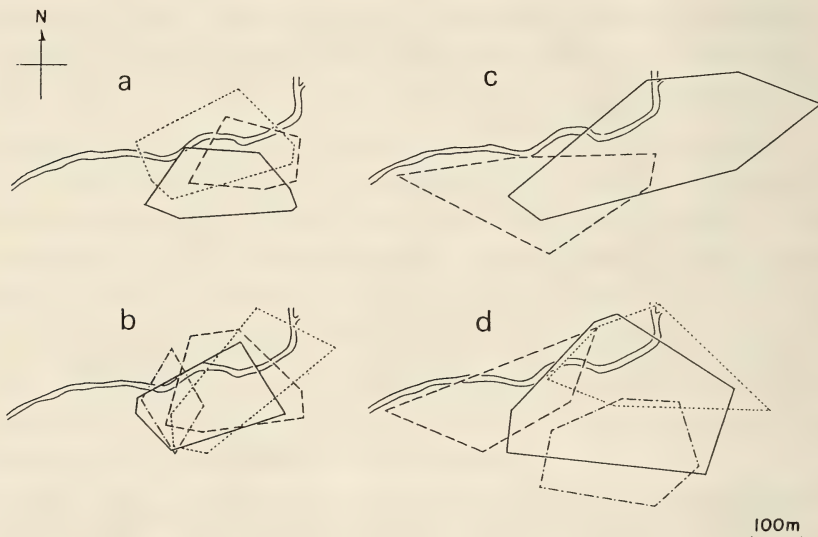
Fig. 6.   The home ranges of three adult male (a), and four adult female (b) *Cynopterus brachyotis*, and of two adult male (c), and four adult female (d) *C. horsfieldi*.
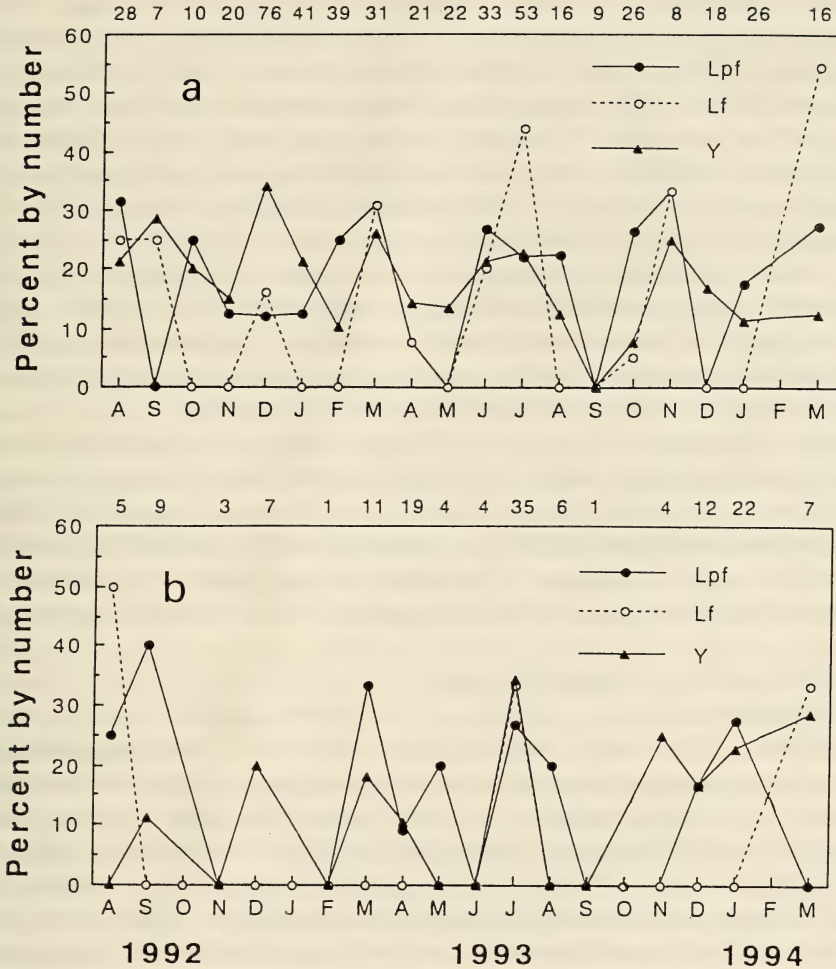
Fig. 7.   Seasonal changes in the composition of late-pregnant females (Lpf), lactating females (Lf) and young (Y) in *Cynopterus brachyotis* (a) and in *C. horsfieldi* (b).   Data are based on the ratios of the number of late-pregnant females, and lactating females, to adult females, and the ratio of the number of young to the total catch every month.   Monthly sample sizes are indicated above graphs.

was heavily pregnant when caught and banded on 17 August 1992, and was lactating when recaptured on 21 December 1992, while female A292, also heavily pregnant when caught on 24 May 1993, was again in the same reproductive condition when recaptured on 8 October 1993.

DISCUSSION

1. Day roosts
   The daytime roosts of *Cynopterus horsfieldi* and *C. brachyotis* are quite

different.   Whereas *C. brachyotis* usually roost in pairs, or in small groups, in trees, under leaves, and occasionally in the twilight areas of caves (Lim 1966, Lekagul and McNeely 1977, Medway 1983, Payne *et al*. 1985, Mickleburgh *et al*. 1992), and in our study area, under fronds near the trunk and beneath the crowns of various trees, *C. horsfieldi* on the other hand is more gregarious and often roosts in shallow caves or rock shelters, and occasionally in trees, especially palms (Lim *et al*. 1974, Medway 1983, Payne *et al*. 1985).   These differences in roost site preference were also noted in the Ulu Gombak study area.   Most *C. brachyotis* roosts were in trees other than palms, while those of *C. horsfieldi* were sparsely distributed because of the small number of palms and the absence of cave or rock shelter roost sites.   Furthermore, *C. horsfield's* pattern of roost site distribution may also result from the scarcity of sites to accommodate their larger size, and larger roost numbers.

Males of both species frequently changed their roost sites, while most of the females rarely changed theirs.   Such differences between the sexes have also been shown to occur in the phyllostomid bat, *Artibeus jamaicensis* (Morrison 1978, Morrison and Handley 1991).   According to Lekagul and McNeely (1977), *C. horsfieldi* and *C. brachyotis* often share roosts, however it remains to be determined just how many individuals typically occur in these aggregations.

2. Activity patterns during the night

The temporal activity patterns of *Cynopterus horsfieldi* and *C. brachyotis* differ greatly.   The initial peak of flight activity of *C. brachyotis* after sunset was one or two hours earlier than that of *C. horsfieldi*.   After the first peak, *C. brachyotis's* activity gradually declined during the night, whereas activity among *C. horsfieldi* decreased around midnight, but then increased again three hours before sunrise.   Radio-tracking data indicate that the smaller *C. brachyotis* is more active during the night than the larger *C. horsfieldi*, with *C. brachyotis* moving around frequently, and with some individuals being captured and recaptured in the same night.   The activity patterns of *C. brachyotis* are somewhat similar to those of *Carollia perspicillata* (Heithhaus and Fleming 1978, Fleming and Heithhaus 1986).   Radio-tracked *C. perspicillata* fed intensively during their first activity period, then settled down to a routine of about one feeding bout per hour (Fleming 1988).   In contrast, the basic activity pattern of *C. horsfieldi* may be bimodal with a resting period around midnight.

3. Food habits and fig seed dispersal

It seems that *C. horsfieldi* has a narrower dietary range than *C. brachyotis*, with the former depending almost entirely on fruits as food (Lekagul and McNeely 1977, Medway 1983, Payne *et al*. 1985, this study), while the latter eats a wide range of fruits weighing 0.4–68.2 g (Boon and Corlett 1989) but also takes flowers, nectar, pollen, leaves and insects (Lim 1970, Medway 1983, Marshall 1985, this study).   Among frugivorous New World Phyllostomid bats there is a high correlation between body weight and the weight of fruits carried away (Bonaccorso 1979).   This correlation is also apparent when examining *C.*

horsfieldi and *C. brachyotis*, with the larger former species carring off fruits averaging 17.8 g, whereas the smaller latter species only carries off fruit averaging 7.9 g. Such size differences may play an important role in the partitioning of food resouces among similar species of fruit bats occurring sympatrically.

In our study, the average distance between fruiting trees and feeding sites was 50–78 m, while in a young secondary forest in Singapore it was within 100 m (Boon and Corlett 1989). As the home ranges of *C. horsfieldi* are larger than those of *C. brachyotis*, we assume that *C. horsfieldi* transport figs further from foraging sites than do *C. brachyotis*, though we have insufficient data to prove this. In our study area, the shortest distance between *Ficus variegata* trees with heights of 30 m or more averaged only 35 m. *Cynopterus* were the most frequently mist-netted bats in the Ulu Gombak study area. Of the 754 fruit bats of nine species captured, 87.1% were *Cynoptrus* species. Similarly, *Cynopterus* comprised about 70% of the fruit bats captured at Bangi, a frag-mented secondary forest site, but only 39% at Kuala Lompat, a primary forest site (Zubaid 1993, 1994). Thus it seems that *Cynopterus* species predominate in secondary forest. In addition to the suite of frugivores birds and arboreal mammals to be found in forests, frugivorous bats such as these *Cynopterus* species are likely to be important seed-dispersal agents for fig trees, enabling such trees to quickly invade a gap or disturbed forest.

4. Home ranges

In both species, the greatest movements measured equalled the distances between the day roosts and feeding sites. In the Ulu Gombak study area the mean distance of 295 m moved by *C. brachyotis* was much shorter than by the same species in Philippine submontane rainforest (650 m; Heideman and Heaney 1989). This difference between sites may result from the fact that our 15 day period of radio-tracking was much shorter than the length of time between captures (10–100 days) in the Philippine study, or from the fact that fruiting trees or feeding areas were closer to the day roosts at our site. In addition, home ranges in the Phillippines may have shifted during those periods. The mean distance moved by *C. horsfieldi* was significantly further than that of *C. brachyotis*.

The home ranges of individuals of both species overlapped, suggesting that neither roosting sites nor food resources were limiting, and thus eliminating the need for the bats to hold territories. The estimated home range sizes may, however, be somewhat smaller than the actual sizes, because of the short periods of radio-tracking. Whereas Heideman and Heaney (1989) estimated that the population density of *C. brachyotis* in primary submontane forest on Negros Island was only 0.2 individuals per hectare, in our secondary forest study area, *C. brachyotis* densities were very high (Funakoshi and Zubaid, unpublished) and home ranges were very small. Such high densities at Ulu Gombak may be associated with the abundance of roost and food resources.

5. Reproductive cycles

Pregnant female *C. brachyotis* and *C. horsfieldi* have been captured in all months, suggesting that breeding is non-seasonal (Lim 1970, Medway 1983). Lim (1970) found that peaks in pregnancies among *C. brachyotis* occur in January, May, and September at the same latitude in Malaysian rainforest as our study area. Such seasonal peaks differ, however, from those in our Ulu Gombak study area where the timing of peaks of pregnancy and lactation vary from year to year. As for the effects of environmental factors on reproduction, Lim (1970, 1973) found that the highest frequency of pregnancy coincided with the greatest availability of fruits.

In the Ulu Gombak study area, it seems that female *C. brachyotis* may produce two or three young each year. This assumption is based on the main pregnancy and lactation peaks (Fig. 7a), and the fact that female A292 had just one reproductive cycle between late May and early October, and female A005 had the opportunity to produce two young between mid August and mid December. On the Philippine island of Luzon, at 14°N, *C. brachyotis* reproduce seasonaly with two birth periods per year (Ingle 1992). On Negros Island (9°22′ N), the length of gestation in *C. brachyotis* is approximately four months, and lactation lasts for about 6-8 weeks (Heideman 1987). At lower latitudes, such as at our study area, both gestation and lactation periods may be shorter because of the short reproductive cycle at Ulu Gombak. Most female *C. brachyotis* become pregnant at about 6-8 months of age, while males become sexually mature at about one year old (Heideman 1987, Mickleburgh *et al.* 1992). In *C. horsfieldi*, the peaks of pregnacies occurred in 4 to 6 month intervals, with most females probably producing two young per year (Fig. 7b). The ages of sexual maturity of this species, however, remain unknown. The relatively short reproductive cycle of female *C. brachyotis* may be one of the factors contributing to the greater size of their populations.

In conclusion, both *C. brachyotis* and *C. horsfieldi* are abundant in partially disturbed rainforest, and can coexist in the same habitat through differences in roost site selection and partitioning of food resources in relation to their different body sizes. *Cynopterus brachyotis* predominates, probably because of the abundance of roost sites and food resources and its more rapid rate of reproduction with two or three litters per year.

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ERRATUM

The following table was omitted from Asada and Ochiai's paper in Mammal Study 21(2) and should have been inserted on page 157.

Table 1. Number of sika deer of conceiving before and after mid October on Boso Peninsula, central Japan.

Conception periods	Maternal age			Total
	1-year-old	2 and 3-year-old	4-year-old and more	
Sep. to mid. Oct.	10	47	91	148
Late Oct. to Dec.	2	11	7	20
Total	12	58	98	168

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The functional anatomy of the masticatory muscles of the Malayan pangolin, *Manis javanica*

Hideki ENDO, Isao NISHIUMI, Masamichi KUROHMARU[1], Jarujin NABHITABHATA[2], Tanya CHAN-ARD[2], Nivesh NADEE[3], Srihadi AGUNGPRIYONO[4], and Junzo YAMADA[5]

Department of Zoology, National Science Museum, Tokyo, Tokyo 169-0073, Japan
[1]*Department of Veterinary Anatomy, Faculty of Agriculture, The University of Tokyo, Tokyo 113-8657, Japan*
[2]*Natural Science Research Division, National Science Museum, Thailand, Bangkok 10900, Thailand*
[3]*Energy and Environment Research Department, Thailand Institute of Scientific and Technological Research, Bangkok 10900, Thailand*
[4]*Department of Veterinary Anatomy, Bogor University of Agriculture, Bogor 16152, Indonesia*
[5]*Department of Veterinary Anatomy, Faculty of Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan*
Fax. +81-3-3364-7104

Abstract.   The masticatory muscles of the Malayan pangolin, *Manis javanica*, were observed in dissection, and relative positions of the cranium and the mandible were examined under soft-X ray photographs. The *M. temporalis* was well-developed in the medial area of the zygomatic process of temporal bone. The *M. masseter* was found to consist of three large well-developed bundles between the zygomatic arch and the mandible. Based on these observations, it is suggested that the thin V-shaped mandible may act as a substantial support in the ventral portion of the oral cavity, and that the *M. masseter* and *M. temporalis* may serve to help fix the shape of mouth, when the pangolin uses the specialized tongue for feeding. We demonstrated that the *M. digastricus* is at least functionally able to depress and open the mandible. In addition, the well-developed *M. mylohyoideus* may contribute to the control of intraoral pressure during mastication.

Key words : digastric muscle, mandible, masseter muscle, pangolin, temporal muscle.

Although the tongue structure of pangolins has attracted the interest of a number of anatomists (Ehlers 1894, Edgeworth 1923, Sonntag 1923, 1925, Lubosch 1938, Kubota *et al*. 1962, Saban 1968, Doran and Allbrook 1973, Yen 1984, 1985, Chan 1995), who have pointed out that the Manidae species use their uniquely elongated tongue for feeding on termites and ants, the morphology of the masticatory muscles and of the mandibular bone of the pangolins has been overlooked. The mastication system has so far been considered functionally vestigial or insignificant (Doran and Allbrook 1973, Walker 1991, Chan 1995), but without there having been detailed descriptions of the masticatory muscles.

The purpose of this study therefore was to examine macroscopically the three dimensional relationship between the cranium and the mandible, and the development of the masticatory muscles in order to clarify their functional significance.

MATERIALS AND METHODS

One formalin-fixed head and three skulls of the Malayan pangolin, *Manis javanica* that had been stored in Thailand Institute of Scientific and Technological Research, in the Department of Veterinary Anatomy of The University of Tokyo and in the Department of Zoology of National Science Museum, Tokyo were used in this study.

The skin, subcutaneous tissue and globe were removed from the fixed head, then the masticatory muscles, *Musculi masseter, digastricus, temporalis, pterygoidei* and *Musculus mylohyoideus* were observed macroscopically. Soft-X ray photographs were taken to examine the articulation and the positional relationship between the mandible and the cranium, and the areas of attachment of the masticatory muscles on the skulls were observed. The anatomical nomenclature of the muscular system was based on Miller's Anatomy of the Dog (Evans 1993).
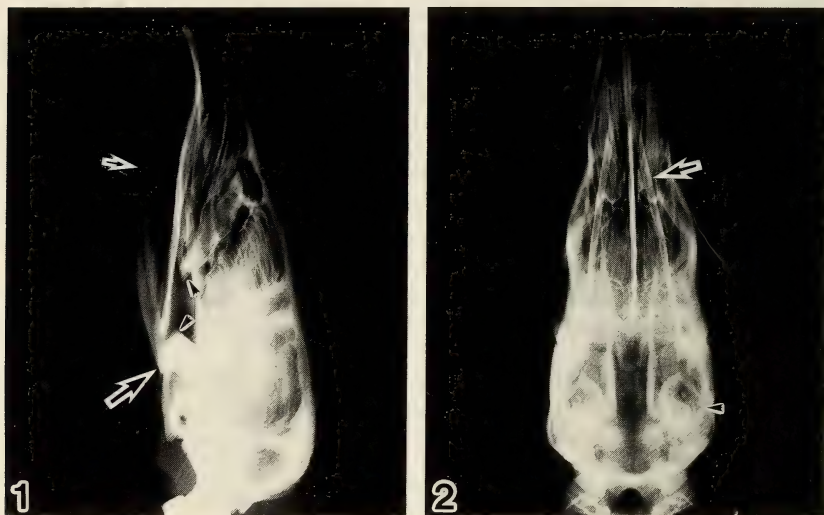
Fig. 1. Lateral soft-X ray photograph of the head of a Malayan pangolin. Rostral direction at the top. The thin mandible (small arrow) is gently curved and connects to the ventro-caudal area of zygomatic process of the temporal bone (large arrow). Arrowheads, incomplete zygomatic arch.

Fig. 2. Dorso-ventral soft-X ray photograph of the head of a Malayan pangolin. Rostral direction at the top. The thin mandible represents the V-shape (arrow). Arrowhead indicates the auditory bulla. The atlas vertebra is present in this specimen.

RESULTS

The relative positions of the mandibular bones and the cranium were observed using soft-X ray photographs (Figs. 1, 2). The mandibular body was found to be slender and gently curved dorso-ventrally, but was not generally well-developed (Figs. 3, 4). The symphysis was relatively long and strong in comparison with the thin mandibular bone. The lateral surface of the mandible was flat and lacked processes for the insertion of muscles, while the medial side had a shallow groove to which *M. mylohyoideus* was attached. The mandible connected to the ventro-caudal area of the zygomatic process of the temporal bone. The articulation area was slightly depressed, and the zygomatic process had no specialized surfaces for articulation (Figs. 3, 4). All three skulls and the preserved head possessed incomplete zygomatic arches which varied in their developmental state (Figs. 1–4). The temporal bone was well-developed dorso-rostrally in the area of the zygomatic process (Fig. 3), which we have called the "temporal-muscle process". The orbit was surrounded by depressed frontal and developed temporal bones, and there was a deep hollow in the caudal part of the orbit.

Fig. 3. Left side of the skull of a Malayan pangolin. Rostral direction at the top. The mandible has been artificially attached to the cranium. The skull is elongated and simple in lateral view, while the mandible bone is slender. The zygomatic arch is not developed (arrows). The temporal bone is dorso-rostrally well-developed in the part of zygomatic process (arrowhead). The depressed orbit is surrounded by the temporal muscle process in the caudal part.

Fig. 4. Ventral view of the skull specimen of a Malayan pangolin. Rostral direction at the top. The mandible articulation area is slightly depressed (arrow).
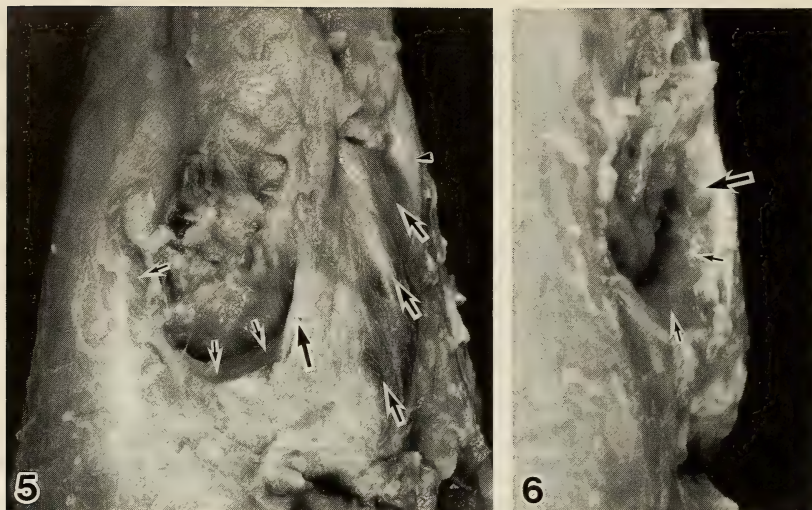
Fig. 5.   Right side of the head of a Malayan pangolin.   Rostral direction at the top.   The wedge-shaped *M. temporalis* is well-developed in the caudal part of the orbit and in the medial side of the temporal-muscle process of the temporal bone (small arrows).   The *M. masseter* consists of three main bundles reaching from zygomatic arch to the caudal part of the mandible (intermediate arrows).   Large arrows, zygomatic arch.   Arrowhead, mandible.

Fig. 6.   Dorso-lateral view of the orbit region of a Malayan pangolin.   Rostral direction at the top.   The wedge-shaped *M. temporalis* is well-developed in the medial side of the zygomatic arch (small arrows).   The large arrow indicates a part of the *M. masseter*.

The *M. temporalis* was well-developed on the medial side of the zygomatic arch of the temporal bone and the temporal muscle process (Figs. 5, 6).   The *M. temporalis*, which was found to be wedge-shaped, was largely attached to the caudal part of the orbit, and was rostrally extended to the medial surface of the zygomatic arch.   The muscle was inserted vertically into the caudal mandible body.   The *M. masseter* consisted of three well-developed main bundles (Figs. 5, 7).   The two cranial bundles originated form the medial side of the zygomatic arch and the most caudal bundle arose from the ventral part of the arch.   All three bundles reached the caudal half of the mandible laterally (Fig. 7).

The *M. digastricus* consisted of two thin parts, the lateral part originating from the ventral area of temporal and occipital bones inserting into the ventral edge of the mandible (Figs. 7, 8), while the thinner medial part arose from the ventral surface of the *M. mylohyoideus* (Fig. 7), and did not attach to the caudal part of the mandible.   The *M. mylohyoideus* was thick and occupied the space between the mandibular bones, and provided an area of attachment for the medial portion of the *M. digastricus* (Fig. 8).

The *M. pterygoideus lateralis* was found to consist of two small, short bundles lying parallel and rostro-laterally oriented from the palatine bone to the medial side of the mandible (Fig. 9).
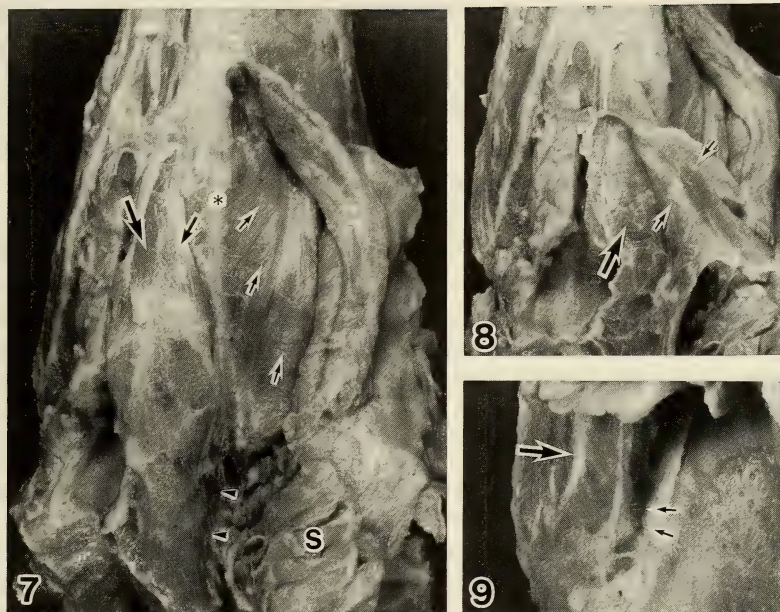
Fig. 7. Ventro-lateral view of the head of a Malayan pangolin. Rostral direction at the top. Superficial muscles are removed. The *M. masseter* consists of three main bundles (small arrows). The *M. digastricus* can be seen. The lateral part of the *M. digastricus* (intermediate arrow) originates from the ventral area of the temporal and the occipital bones (arrowheads), while the thinner medial portion (large arrow) rises form the ventral surface of the *M. mylohyoideus*. Asterisks, the ventral edge of the mandible. S, submandibular gland.

Fig. 8. Ventro-lateral view of the head of a Malayan pangolin. Rostral direction at the top. The *M. digastricus* is turned out, and the two distinctive parts can be observed (small arrows). The *M. mylohyoideus*, which is thick and occupies the space between mandibles (large arrow).

Fig. 9. Ventral view of the head of a Malayan pangolin. The *M. pterygoideus lateralis* consists of two small and short bundles (small arrows). The large arrow indicates the ventral edge of mandible to which the *M. digastricus* is attached. Rostral direction at the top.

DISCUSSION

The possibility of morphological differences between species of Manidae in the developmental of the masticatory muscle should be taken into account, particularly given that previous descriptions have not been consistent (Lubosch 1938, Saban 1968, Yen 1985, Chan 1995).

Firstly, it is suggested that the thin V-shaped mandible may provide significant support for the ventral portion of the oral cavity. It became clear during this study of the Malayan pangolin that the *M. temporalis* was developed and had the enlarged attachment to the temporal-muscle process. The multi-bundled *M. masseter* was also found to be a strong mastication motor. Although the developmental state of the zygomatic arch was found to vary between individuals, we believe, contrary to Saban (1968), that the arch is not

vestigial.   Although active movement of mandible is certainly not important for feeding in this species, we suggest that the zygomatic arch and its temporal-muscle process, the *M. masseter* and *M. temporalis*, may all serve to help fix the shape of mouth.   Although the mandible is simple and thin, the symphysis is relatively well-developed in the pangolins (Lubosch 1938, Saban 1968) indicating that mandibular bones may support the shape of the oral cavity while feeding with the tongue.

In comparison with the *M. masseter* and *M. temporalis*, the *M. digastricus* of the Malayan pangolin is not comparable with that of other mammals (Edgeworth 1923, 1935, Evans 1993).   Although Chan (1995) pointed out that the *M. digastricus* disappears into the submandibular gland, in this study we have demonstrated that the lateral part of the *M. digastricus* is at least functional in depressing and raising the mandible.   It is further suggested that the medial part of the *M. digastricus* only assists the action of the well-developed *M. mylohyoideus*.   The *M. mylohyoideus* may support the function of *M. digastricus* and act as a depressor of the mandible.   In addition, the well-developed *M. mylohyoideus* may contribute to the control of intraoral pressure during mastication.   Specimens with intact hyoid bones should be examined morphologically in the future to elucidate this.

Our description of *Mm. pterygoidei* is similar to that of Yen (1984).   The *M. pterygoideus lateralis* could not be confirmed in this specimen.   It remains unclear how this muscle has changed in form and function.

The functional significance of masticatory muscles of certain rodents has been described (Kesner 1980, Bekele 1983, Druzinsky 1995), and functional models of mandibular movement have also been established for some rodents (Weijs 1975, Gorniak 1977, Byrd 1981, Satoh 1997).   On the basis of data from *Apodemus* and *Clethrionomys* species (Satoh 1997), it has been suggested that patterns of mandibular movement are directly modified by adaptations in dental morphology.   We speculate, however, that the masticatory muscles in toothless mammals such as the pangolin have also been functionally affected by their special feeding pattern.   In such mammals, the primary function of masticatory muscles may not be to generate occlusal force, but to control the air pressure within the oral cavity.

In contrast with previous speculations (Doran and Allbrook 1973, Chan 1995), the present study has clearly demonstrated that the masticatory muscles of the Malayan pangolin are not vestigial, but functional, well-developed, fix the mandibular bones, support the shape of the oral cavity, and help control the pressure in the oral cavity during feeding with the tongue.

The masticatory muscles of other toothless mammals may also be a functional part of the mastication system and may also have been adapted for special feeding as a form of functional convergence.

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      muscles and their spindle supply in the pangolin.   J.Stomatol. Soc. Japan 51 : 674−688.
Yen, Y. C. 1985.   Comparative studies on characteristics structures of sensory and motor mechanisms
      in the stomatognathic system of the pangolin, *Manis aurita* (Mammalia) : 2.   Electron micro-
      scopic observations on the masticatory muscle spindles in the pangolin.   J. Stomatol. Soc.
      Japan 52 : 16−43.

Histochemical properties of the masticatory muscles of murids

Katsumi Sugasawa and Takayuki Mōri*

Zoological Laboratory, Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812-81, Japan
Fax : +81-92-642-2804, e-mail : sugar@agr.kyushu-u. ac. jp

Abstract.　Histochemical studies were made of the masticatory (temporal, masseter and digastric) muscles of the laboratory mouse, *Mus musculus*, and laboratory rat, *Rattus norvegicus*, which are omnivorous, the golden hamster, *Mesocricetus auratus*, which is omnivorous but with a tendency to eat much vegetable matter, and the Japanese field vole, *Microtus montebelli*, which is herbivorous. It was found that the masticatory muscles were composed almost entirely of fast-twitch fibers.　Interspecific differences were found in the oxidative enzyme activity of the masseter muscle in relation to rodent dietary habits.　The masseter muscles of the mouse and rat consisted of fast-twitch oxidative glycolytic and fast-twitch glycolytic fibers, thus they appear to have the capacity for powerful, or sudden, and enduring contractions.　The masseter muscles of the hamsters were composed only of fast-twitch intermediate fibers, thus giving them the capacity for moderately enduring contractions, whereas the vole masseter muscles consisted only of fast-twitch oxidative fibers, and consequently they appear to have the capacity for particularly enduring contractions.

Key words : fiber types, food habits, histochemistry, masticatory muscles, murids.

The wide range of mechanical demands imposed upon the masticatory apparatus of mammals is reflected in its structural and functional diversity.　Despite detailed descriptions of skull and mandible anatomy, and analyses of patterns of jaw movements and coincident muscle activity in a wide variety of mammals (De Gueldre and De Vree 1988), little is known about the histochemical characteristics of the masticatory muscle fibers themselves.

　　Studies of the masticatory muscles of laboratory animals (Taylor *et al.* 1973, Schiaffino 1974), livestock (Suzuki 1977), and man (Ringqvist 1973, 1974) have demonstrated that, like most limb muscles, these muscles are generally heterogeneous with respect to fiber type.　Other studies have shown that the masticatory muscles have regional differences in fiber length and fiber distribution, and that these structurally different regions imply different patterns of muscular activity (Herring *et al.* 1979, Maxwell *et al.* 1979, Gorniak 1985).

　　Carnivores chew rapidly, have blade-like dentition for slicing, show little

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*To whom correspondence should be addressed

horizontal jaw movement, possess a large temporalis and relatively small masseter complex, and have a high percentage of fast-twitch fibers in their masticatory muscles. In contrast, herbivorous animals, such as cows, sheep and rabbits, chew relatively slowly, have flat teeth for grinding, show extensive horizontal movements of the lower jaw, possess a large masseter complex and relatively small temporals, and have a high percentage of slow twitch fibers within their masticatory muscles.

The characteristic specialization of the Rodentia is their ability to use their incisors to gnaw hard fibrous substances. Gnawing is possible because their large upper and lower incisors grow continuously. In rodents, both gnawing and chewing involve predominantly anteroposterior (*propalinal*) movements of the mandible. The characteristic structural modifications of rodent mandibles and masticatory muscles are all related to this *propalinal* movement.

Although a number of functional studies have been made on mammalian masticatory muscles, few studies have focussed on the relationships between the histochemical characteristics of these muscles and the food habits of rodents.

The purpose of the present study is to clarify aspects of adaptation for particular feeding habits in murids by comparing the histochemical characteristics of the masticatory muscles of the omnivorous mouse, *Mus musculus* (Cunliffe-Beamer and Les 1986) and rat, *Rattus norvegicus* (Weihe 1986), the omnivorous golden hamster, *Mesocricetus auratus* which tends to eat a great deal of vegetable matter (Hobbs 1986), and the fully herbivorous Japanese field vole, *Microtus montebelli*.

MATERIALS AND METHODS

Five adult laboratory mice, four laboratory rats, three golden hamsters, and eight Japanese field voles were euthenased for this study. The anterior part of the temporal muscle, *Musculus temporalis*, the superficial masseter muscle, *M. masseter superficialis*, and the anterior belly of the digastric muscle, *M. digastricus*, were all removed.

For light microscopy, muscle tissues were rapidly frozen in isopentane solution cooled with dry ice. Serial cross-sections of the muscles, 8 μm thick, were obtained and stained : for myosin adenosine triphosphatase (ATPase) (Padykula and Herman 1955) after alkaline (pH 10.5) or acid (pH 4.3) pre-incubation (Brooke and Kaiser 1970a, b, Suzuki 1977) ; for reduced nicotinamide adenine dinucleotide dehydrogenase (NADH-DH) (Burstone 1962), and for phosphorylase activities (Takeuchi and Kuriaki 1955).

Fibers were classified as either : slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), fast-twitch intermediate (FI) with intermediate NADH-DH activity between FOG and FG, and fast-twitch oxidative (FO) on the basis of their differences in their reactivity for myosin ATPase after alkali and acid pre-incubation, and activity for NADH-DH and phosphorylase (based on Peter *et al.* [1972] and Armstrong *et*

Table 1. Histochemical enzyme activities of myofiber types in the masticatory muscles of the mouse, rat, hamster and Japanese field vole, *Microtus montebelli*. −, Unreactive ; +, weak ; ++, modemate ; +++ to ++++, strong. For myofiber type, see in the text.

Myofiber type	Myosin ATP ase		NADH-DH	Phosphorylase
	pH 10.5	pH 4.3		
SO	−	+++	+++	+
FOG	++++	−	+++ to ++++	++ to +++
FG	++++	−	+	+++
FI	++++	−	++	++
FO	++++	−	+++++	+

al. [1977], see Table 1).

The sizes of the muscle fibers concerned were determined by measuring the maximum distance across the lesser diameter of 50 fibers (Brooke 1970) of each type on photographs ($\times 1,000$) using sections stained for myosin ATPase after alkaline pre-incubation.   Means and standard deviations of the diameter were calculated.

RESULTS

In the mouse, the temporal, masseter (Figs. 1a, b, c) and digastric muscles were composed of 35-46% FOG and 54-65% FG fibers (Table 2).   Concerning NADH-DH activity of the mouse masseter muscle FOG fibers, diformazan

Table 2.   Percentages (means ± *SD*) of myofiber types in the masticatory muscles of the mouse, rat, hamster and Japanese field vole, *Microtus montebelli*. The numbers of animals analyzed are given in parentheses.

Animal Muscle	Myofiber types (%)				
	SO	FOG	FG	FI	FO
Mouse (5)					
Temporal	0	46.0±5.9	54.0±5.9	0	0
Masseter	0	34.9+3.1	65.1±3.1	0	0
Digastric	0	36.7±4.0	63.3±4.0	0	0
Rat (4)					
Temporal	0	30.0±5.8	70.0±5.8	0	0
Masseter	0	41.9±3.7	58.1±3.7	0	0
Digastric	0	39.2±5.2	60.8±5.2	0	0
Hamster (3)					
Temporal	0	13.0±6.3	87.0±6.3	0	0
Masseter	0	0	0	100±0	0
Digastric	0	50.1±8.3	49.9±8.3	0	0
Vole (8)					
Temporal	0	541.52	0	45.9±5.2	0
Masseter	0	0	0	0	100±0
Digastric	9.1±3.8	0	0	91.0±3.8	0

deposits were larger than those of the rat.   In mouse masticatory muscles, the diameter of the FOG fibers in the temporal muscles was smallest (20.37±4.27 μm), while the diameter of the FG fibers in the digastric muscles was largest (42.39±3.84 μm) (Table 3).

In the rat, the temporal, masseter (Figs. 2a, b, c) and digastric muscles were composed of 30-42% FOG and 58-70% FG fibers (Table 2).   NADH-DH activity of the FOG fibers in rat masseter muscles was weak, and diformazan deposits were smaller than those in the mouse.   In rat masticatory muscles, the diameter of the FOG fibers in the masseter muscles was smallest (19.52±2.80 μm), while the diameter of the FG fibers in the digastric muscles was largest (46.38±5.38 μm) (Table 3).

In the golden hamster, the temporal muscles were composed of 13% FOG and 87% FG fibers.   The masseter muscles consisted only of FI fibers which reacted strongly for myosin ATPase after pre-incubation at pH 10.5 (Fig. 3a), did not react at pH 4.3 (Fig. 3b), and reacted intermediately for NADH-DH (Fig. 3c).   In particular, the NADH-DH activity of the FI fibers in the masseter muscles was weak in the sarcoplasm and strong beneath the sarcolemma.   The digastric muscles were composed of 50% FOG and 50% FG fibers (Table 2). The diameter of the FOG fibers in the temporal muscles was smallest in the hamster masticatory muscles (31.12±7.12 μm).   On the other hand, the diameter of the FG fibers in the digastric muscles was the largest among the murid masticatory muscles (68.58±5.99 μm) (Table 3).

In the vole, the temporal muscles were composed of 54% FOG and 46% FI fibers.   The masseter muscles consisted only of FO fibers which strongly reacted for myosin ATPase after pre-incubation at pH 10.5 (Fig. 4a), but which

Table 3.   Diameters (means ± *SD*) of myofiber types in the masticatory muscles of the mouse, rat, hamster and Japanese field vole, *Microtus montebelli*.

Animal	Diameter of each myofiber type (μm)				
Muscle	SO	FOG	FG	FI	FO
Mouse					
Temporal	—	20.37±4.27	34.00±6.68	—	—
Masseter	—	22,00±2,23	37.30±3.24	—	—
Digastric	—	21.50±5.37	42.39±3.84	—	—
Rat					
Temporal	—	21.72±1.96	30.56±3.56	—	—
Masseter	—	19.52±2.80	30.00±3.96	—	—
Digastric	—	21.88±5.91	46.38±5.38	—	—
Hamster					
Temporal	—	31.12±7.12	43.36±6.40	—	—
Masseter	—	—	—	36.80±6.16	—
Digastric	—	42.77±3.76	68.58±5.99	—	—
Vole					
Temporal	—	19.41±3.26	—	20.40±2.98	—
Masseter	—	—	—	—	18.00±3.13
Digastric	8.70+1.74	—	—	—	22.72+4.17
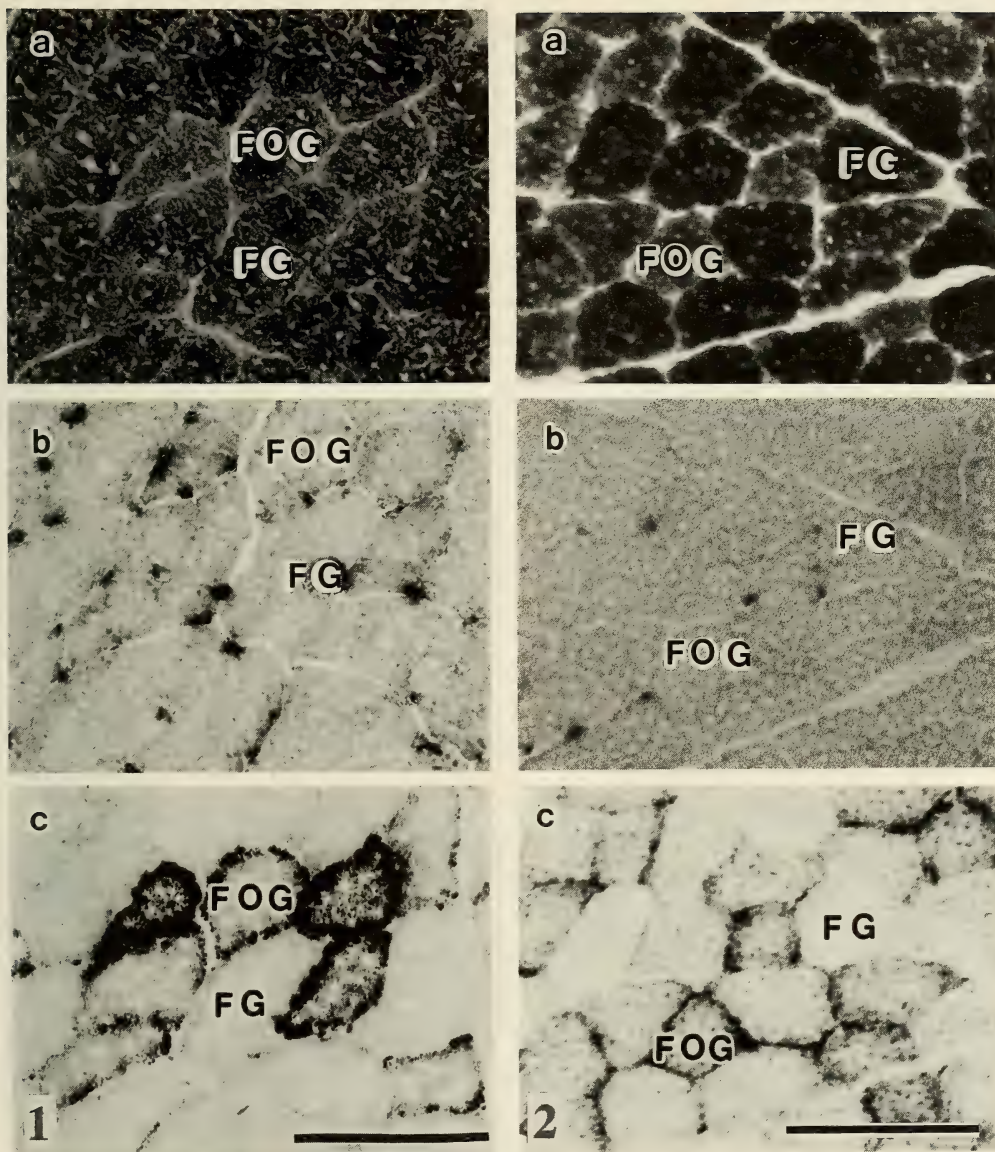
Fig. 1. Histochemical profiles of the masseter muscles in the mouse. a : myosin ATPase activity at pH 10.5, b : myosin ATPase activity at pH 4.3, c : NADH-DH activity. FG : fast-twitch glycolytic fiber, FOG : fast-twitch oxidative glycolytic fiber. Bar : 100 μm.

Fig. 2. Histochemical profiles of the masseter muscles in the rat. Explanations for a, b and c are the same as for Fig. 1. FG : fast-twitch glycolytic fiber, FOG : fast-twitch oxidative glycolytic fiber. Bar : 100 μm.
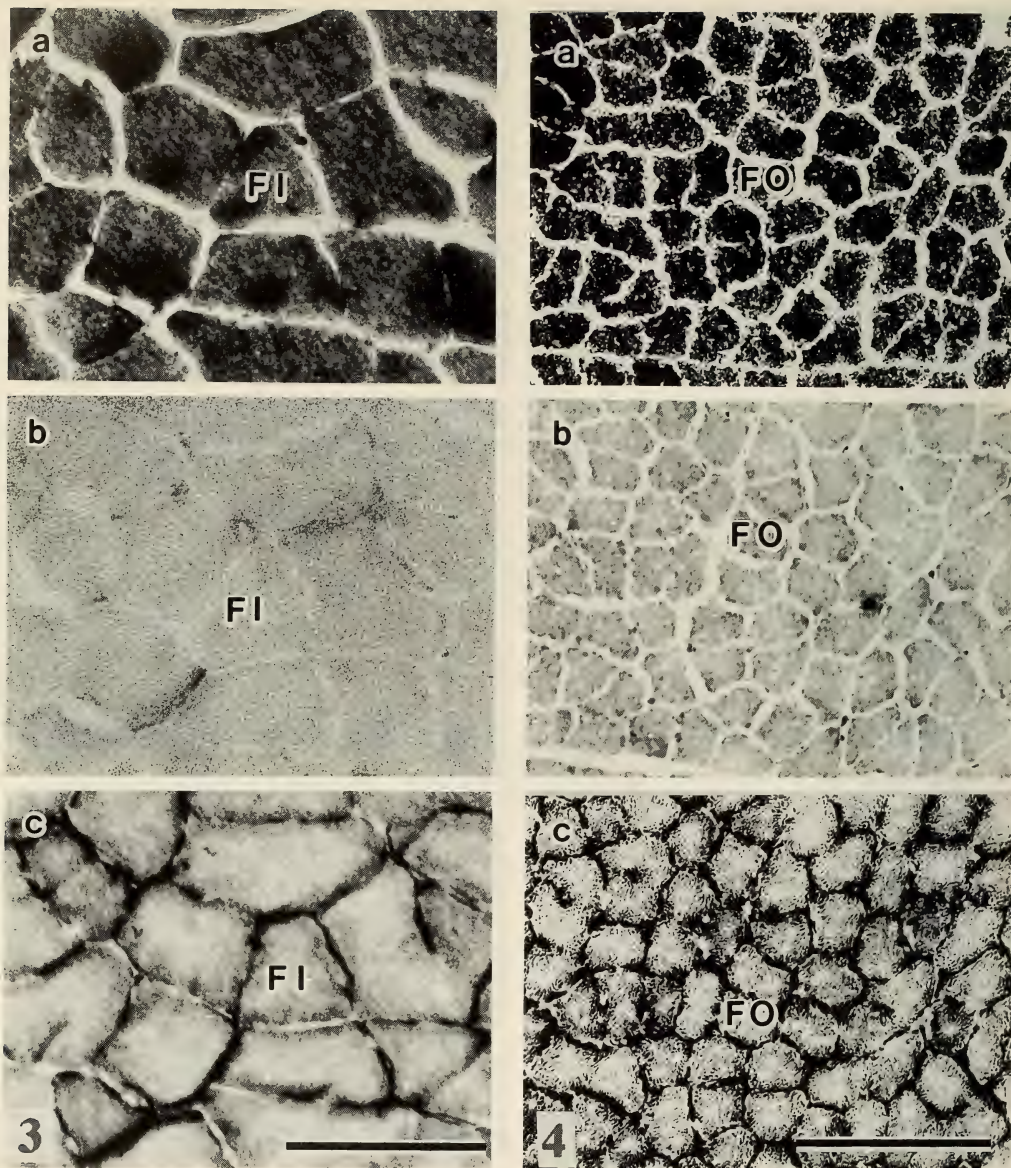
Fig. 3. Histochemical profiles of the masseter muscles in the hamster. Explanations for a, b and c are the same as for Fig. 1. FI : fast-twitch intermediate fiber. Bar : 100 μm.

Fig. 4. Histochemical profiles of the masseter muscles in the vole. Explanations for a, b and c are the same as for Fig. 1. FO : fast-twitch oxidative fiber. Bar : 100 μm.

did not react at pH 4.3 (Fig. 4b), although they reacted strongly for NADH-DH (Fig. 4c). Large granular diformazan deposits and a strong reaction in the subsarcolemmal region for NADH-DH were recognized in the masseter muscle fibers. The digastric muscles were composed of about 10% SO, and about 90%

FI fibers (Table 2). The diameter of the SO fibers in the vole digastric muscles was the smallest among the murid masticatory muscles ($8.70 +/- 1.74 \mu$m). On the other hand, the diameters of the FOG, FI and FO fibers in the vole masticatory muscles were about 20 μm (Table 3).

DISCUSSION

Adult mammalian skeletal muscles are composed of mixtures of highly specialized fibers in proportions that reflect the muscle's function. As for the muscle fiber types found in this study, it was previously well known that : small diameter SO fibers predominate in continuously active muscles that generate low force ; FOG fibers are found in muscles capable of maintaining contractile activity with high force ; and large diameter FG fibers are found in muscles involved in phasic bouts of very high force (Pette and Vrbova 1985). Although, according to Pette and Staron (1997), IIA fibers do not necessarily equate to FOG fibers, vole masseter muscle FO fibers, with strong oxidative activity, may be classified as FOG (IIA) subtype, because the FO fibers of the pectoral muscles of the bat, *Myotis lucifugus*, are composed just of rat IIA myosin heavy chains (Hermanson *et al.* 1991). Thus, the FO fibers of vole masseter muscles also seem to be extremely specialized for fast and sustained contraction. The FI fibers appear to correspond to IIX fibers characterized by an aerobic oxidative capacity intermediate between those of FOG (IIA) and FG (IIB) fibers according to Pette and Staron (1990).

Most information obtained to date on the histochemistry of fiber composition of masticatory muscles in mammals indicates that they are of a heterogeneous nature, and that they vary considerably in the proportion and cross-sectional area of each fiber type both within and among species (Suzuki 1977, De Gueldre and De Vree 1991, Hurov *et al.* 1992, Miyata *et al.* 1996). Such interspecific variation may be due to differences in feeding specializations among mammals. The movement of the jaw during the feeding cycle is relatively complex, and differentiation in muscle fiber composition among the masticatory muscles reflects the different functions that they play during the feeding cycle.

The murid masticatory muscles examined in this study were composed almost entirely of fast-twitch fibers, seeming to imply that murids can quickly masticate various types of food.

The temporal muscles facilitate the powerful upward movement of the mandible (Hiiemae and Houston 1971). In the omnivorous mouse, rat and hamster, these were composed of 13-46% FOG and 54-87% FG fibers, thus giving them the capacity for powerful, or sudden, and enduring contractions suitable for gnawing. The temporal muscles of the hamster contained the most FG fibers indicating that of the species studied, they excelled in phasic bouts of very high force. In contrast, since the temporal muscles of the herbivorous vole consist of 54% FOG, and 46% FI fibers, they have a more enduring contractile ability than either the mouse, rat or hamster.

The masseter muscle, which protracts and elevates the mandible (Hiiemae and Houston 1971), is the largest masticatory muscle in rodents. The histochemical properties of this muscle in rodents are controversial, because this muscle contains various proportions of fiber types (Mao *et al*. 1992). The masseter muscles of the rat were composed of both FOG and FG fibers. These findings confirmed Miyata *et al*.'s (1993) observations of rat masseter muscles. Since the masseter muscles of the mouse and rat consisted of 35-42% FOG and 58-65% FG fibers, they appeared to have the capacity for powerful, or sudden, and enduring contractions suitable for chewing. The masseter muscle of the hamster was composed entirely of FI fibers, thus it seemed to have a greater capacity for enduring contractions than those of either the mouse or the rat. On the other hand, as pointed out by Sugasawa *et al*. (1997), the masseter muscle of the vole consisted only of FO fibers with a remarkably enduring contractile ability, indicating that among the murids studied here, the vole's masseter muscle appears to be best adapted for masticating coarse fibrous materials.

The digastric muscles, which serve to retract the mandible (Woods 1975), were found to be composed of FOG and FG fibers in both the mouse and the rat, confirming Hurov *et al*.'s (1992) findings for the mouse and Kiliaridis *et al*.'s (1988) findings for the rat. Furthermore, since the digastric muscles of the mouse, the rat and the hamster were composed of 37-50% FOG and 50-63% FG fibers, they were able to open their mouths rapidly.

In contrast, the vole, with digastric muscles consisting of 91% FI fibers, is better suited for enduring contractions, than the other murids.

In conclusion, murid masticatory muscles are composed almost entirely of fast-twitch fibers, enabling them to masticate quickly. The masticatory muscles of herbivorous voles have enduring contractile ability, while those of omnivorous murids have powerful or sudden contractile ability. Such a tendency was particularly reflected in the histochemical properties of the masseter muscles.

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Regulation of reproduction in a natural population of the small Japanese field mouse, *Apodemus argenteus*

Keisuke NAKATA

Hokkaido Forestry Research Institute, Bibai, Hokkaido 079-0198, Japan
Fax : +81-1266-3-4166, e-mail : nakata@hfri.bibai.hokkaido.jp

Abstract. Changes in reproductive parameters were analyzed quantitatively in a natural population of the small Japanese field mouse, *Apodemus argenteus*. Among individuals born in the current year, the lightest female weighed 8 g at sexual maturity and the lightest male weighed 10 g. Irrespective of season, the lightest mice were found only during the phase of population increase. Among mice that had over-wintered, the lightest individuals of each sex to reproduce during the spring population decline weighed 10 g. In years of high population density, the reproductive rates of females, even at their peak during August, was below 10%. In contrast, in low-density years, much higher rates, over 69%, lasted until October during the increase phase. The patterns for males were almost the same as those of females during survey years. According to multivariate analysis, the reproductive rate of males was largely explained by population density (partial correlation, 0.732), whereas the reproductive rate of females was largely explained by the fluctuation phase (0.848). The number of each sex to reproduce increased in proportion to the density of potentially reproductive mice at lower densities, but then decreased at higher densities. The observed maximun number of reproductively active mice was 15 males and 21 females in a one-hectare grid. Temperature appeared not to cause any variation in the breeding season in this population.

Key words : age at sexual maturity, breeding season, limitation of reproduction, population density, temperature.

In contrast to the considerable amount of information available on arvicoline rodents (*e.g.,* Alibhai and Gipps 1985, Taitt and Krebs 1985), little is known about the multi-annual fluctuations of murine rodent population densities, particularly of *Apodemus* species. In almost all cases reported so far, *Apodemus* populations usually repeat similar seasonal patterns of density change from year to year (*e.g.,* Fujimaki 1969, Watts 1969, Bobek 1973, Nishikata 1979, Flowerdew 1985, Moreno and Kufner 1988, Lin and Shiraishi 1992). These repeating patterns have led to analyses of density variation in relation to extrinsic factors such as seed crops and temperature, which show marked seasonal variation. With the exception of aggression among adult males (see Watts 1969), however, the effects of intra-population or intrinsic factors affect-

ing *Apodemus* species have been little studied.

Reproductive inhibition has been stressed as one of the most critical aspects of population regulation in small rodents.   For example, limitation of the number of reproductively active individuals is corroborated in natural populations of arvicoline rodents, and the limitation is interpreted as a key attribute in explaining delayed maturation in individuals of the year (*e.g.,* Saitoh 1981, Ostfeld 1985, Nakata 1989).

Montgomery (1989) described spatial density-dependence in the reproductive activity of female *Apodemus sylvaticus*, a dependence which he suggests is a likely regulatory mechanism leading to reproductive inhibition. Temporal density-dependence in reproduction, however, has hardly been studied in *Apodemus* species.

The present study was designed, therefore, to make quantitative analyses of : 1) multi-annual density fluctuations, and 2) the parameters of reproductive inhibition in the small Japanese field mouse *Apodemus argenteus*.

MATERIALS AND METHODS

The study was conducted in a natural mixed forest at Mizuho (43°42′N, 142° 39′E), about 25 km east of Asahikawa, in central Hokkaido, Japan.   This semi-boreal forest consists of both coniferous and broad-leaved trees (Tatewaki 1958, Hamet-Ahti *et al.* 1974).   The dominant tree species are *Abies sachalinensis, Picea yezoensis, Cercidyphyllum japonicum, Tilia japonica* and *Acer mono*, and the undergrowth consists mainly of a dense layer of *Sasa senanensis*.   The output of seed from these species appeared to be rather constant from year to year during the years of the study according to local foresters.   Climatic data for the study area can be found in Nakata (1989).

Capture-mark-release studies were undertaken from June 1975 to October 1979 in a trapping grid set at an elevation of about 460 m.   One hundred trap stations were set 10 m apart in a 10 × 10 pattern in this grid.   During 1975, and for two months of 1976, however, the trapping pattern of the grid was changed. A 5 × 6 pattern was used in June 1975, a 7 × 6 pattern in August and October 1975, and a 5 × 10 pattern in May and September 1976.   Two Sherman-type live traps, baited with oats, were set less than one metre apart at each trapping point, and one trapping session was conducted on three consecutive days each month during the snow-free seasons.

Captured mice were sexed, weighed, their point of capture was noted, and their reproductive condition recorded.   Assuming that marked individuals were removed, the size of the population was estimated using Zippin's (1956) methods.   In order to estimate the effective trapping area (Dice 1938), the mean observed range lengths were calculated from mice which were captured three times at two or three different trap points during each trapping session.   The population density per hectare was obtained by dividing the estimated number of mice by the effective trapping area.   The study area and the trapping procedure are described in more detail in Nakata (1986, 1989).

Males with descended testes were regarded as sexually active, while sexually active females were those either visibly pregnant, or with medium or large nipples indicating that they were lactating, or those with perforated vaginae.

. In order to obtain further reproductive data, mice for autopsy were captured from trap lines located 250–500 m away from the live-trapping grid. These trap lines were situated in the same vegetation as the main trapping grid, however some additional trapping sessions were undertaken during months with snow-cover. The following data were recorded from these mice : weight, total length, tail length, length of testes, condition of the epididymal tubules (visible to naked eyes or not : see Jameson 1950), number of embryos, number of placental scars and uterus width. In addition, the development and wear of the third upper molar (M^3) were used as indices of age (Fujimaki 1966).

In order to be able to make comparisons with other rodent studies, a "cyclicity" index was calculated for the population : $s = \sqrt{(\log N_i - \overline{\log N_i})^2 / (n-1)}$, where $\log N_i$ is the log density at the same time each year, $\overline{\log N_i}$ is its mean and n the sample size (Stenseth and Framstad 1980, Henttonen *et al.* 1985). This index is the standard deviation of the log density.

Four phases of the fluctuating population were arbitrarily defined (Fig. 1), these were : 1) the low phase when there were fewer than eight individuals/ha, 2) the increase phase when increase was rapid, 3) the peak, and 4) the decline phase when decrease was rapid. These four phases occurred in rapid succession during a period of less than one year (Krebs and Myers 1974).

In order to assess the effect of temperature on the population (see Fig. 5) temperature records were obtained from the Higashikawa Meteorological Station (alt. 216m) 10 km south-west of the grid. Records of monthly precipitation and snow depth can also be found in Nakata (1989).

RESULTS

1. Density changes

During the course of our five year study, the *Apodemus argenteus* population varied in size, such that some years were high-density, and others were low-density (Fig. 1). In 1976 and 1978, high-density years, the population density increased rapidly from May to July, reached its peak in August, then declined from September onwards. In contrast, in 1977 and 1979, low-density years, the population decreased from May to June or even August, then increased until October. The autumnal increases were not gradual, and they were at similar rates of growth as found during high-density years. The changes in density recorded in 1975 may be somewhat over-estimated as a result of bias caused by the small-scale of trapping at that time, however, despite that, the changing pattern was similar to that observed in both 1977 and 1979.

The highest density recorded during the study was 78.5 individuals/ha in August 1978, whereas the lowest density was 2.8 individuals/ha in August 1977. The amplitude of the change was 28-fold. As described above, a sharp con-
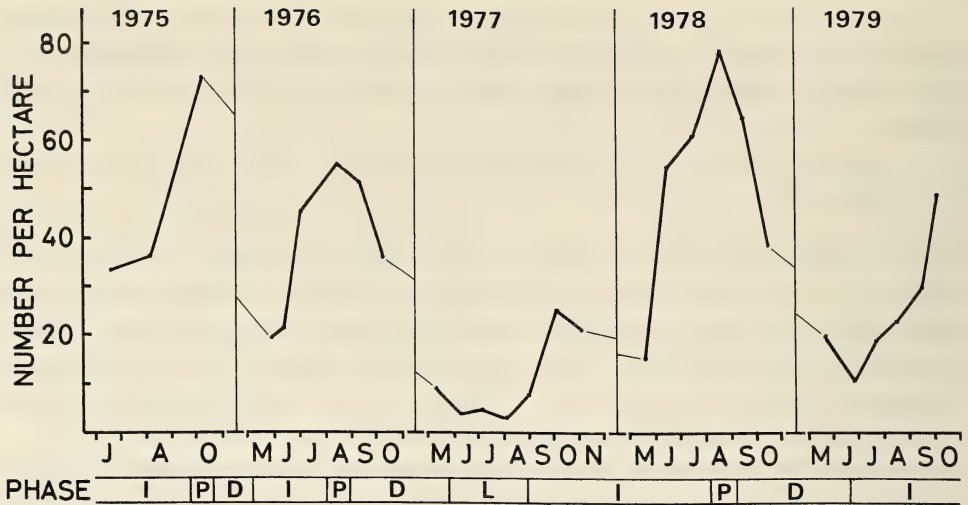
Fig. 1.   Fluctuation in population density of live-tr̊apped *Apodemus argenteus*. L=low phase, I=increase phase, P=peak phase, D=decline phase.

trast was found between different summers and consequently the s-values varied during the five years being 0.453 in June, 0.567 in August and 0.173 in October.

The duration of the increase phase was highly variable, ranging from three months (from September to July in 1976) to eleven months (from September 1977 to July 1978).   The over-winter decline phases were rather longer, namely from September 1976 to May 1977 and from September 1978 to June 1979.   The low phase lasted for three months in 1977, and in 1979 there was no low phase between the decline and increase phases. The population declined just after attaining its peak density, and accordingly the peak phase was regarded as a brief time covering just one month.

2. Age and body weight at reproduction

Among mice of the year, the lightest sexually mature males weighed 10 g (one male in June 1976), and the lightest sexually mature females 8 g (two females in July 1976 and October 1977 ; see Fig. 2).   Irrespective of season, these lightest mice were found only during the increase phase.   Among the autopsied mice, the youngest male was 2-4 months old, and the youngest female was 1-2 months old (Appendix 1).

Among those mice that had over-wintered, the lightest individuals to reproduce weighed 10 g (one male and one female in May 1977, and one female in May 1979), when the population was in the decline phases (Fig. 2).   These light mice were presumably more than eight months old, given that reproductive activity stopped early in the preceding year (no reproductive females were live-trapped either in October 1976, or during September and October 1978). The existence of shortened reproductive seasons was corroborated by the findings from the autopsied samples (Appendix 1).

3. Reproductive rate and the number of reproductively active individuals

Temporal changes in the reproductive rate of *Apodemus argenteus* were examined. In order to quantify the reproductive rate (the percentage of individuals reproductively active), males were considered capable of reproducing
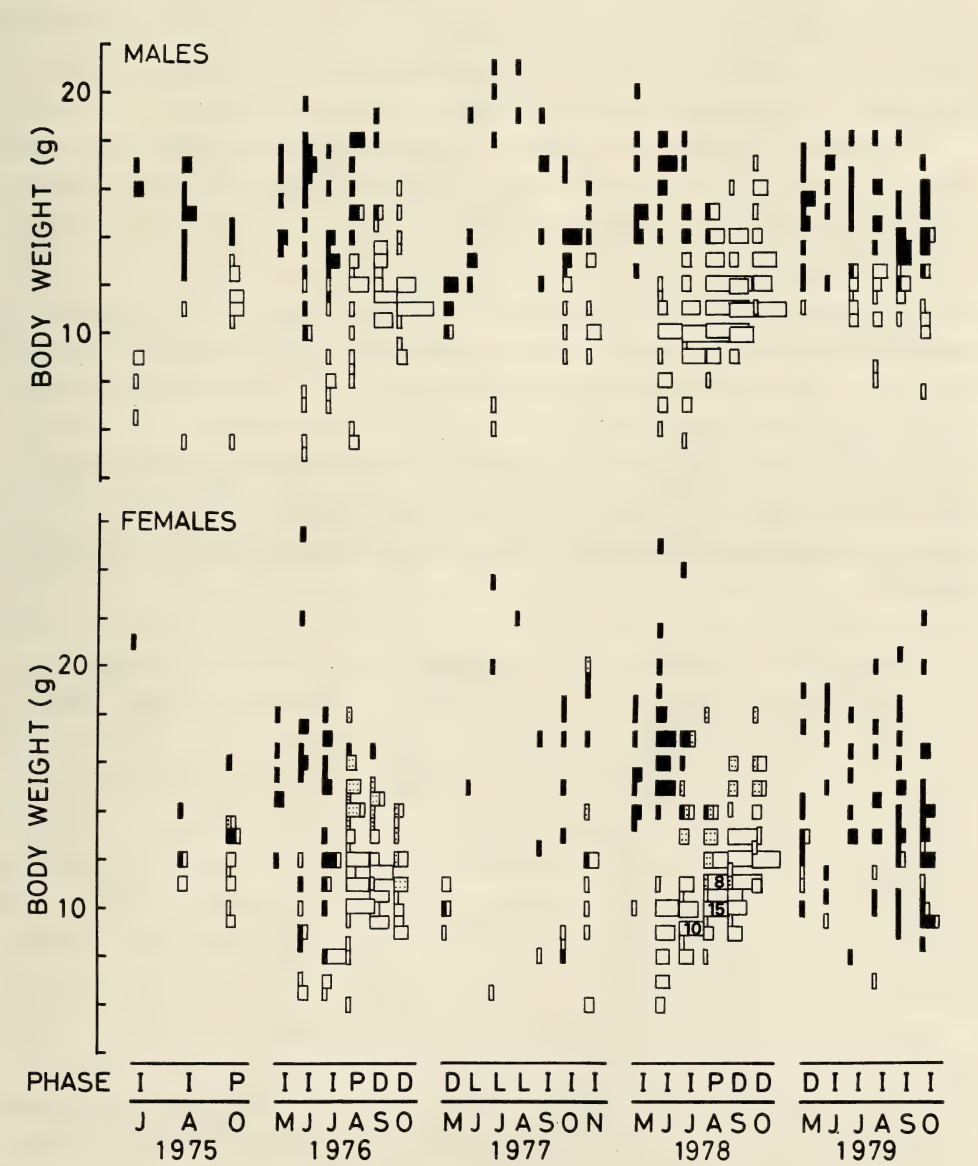
Fig. 2. Body weights of live-trapped male and female *A. argenteus*. Each small rectangle represents one mouse. ■=reproductively active mouse, □=immature mouse including post-reproductive males, ▣=post-reproductive female. Numbers are sample sizes. Other symbols are as in Fig. 1. All mice trapped in May had over-wintered except for one female of the year in 1978 (see text).

when they weighed 10 g or more, and females were considered capable of reproducing when they weighed 8 g or more.

Rates of reproduction were closely associated with fluctuation phase and population density (Fig. 3). The proportion of reproductively active females during the peak phase in August in high-density years was just 1% in 1976 and only 5% in 1978, whereas in low-density years (1977 and 1979) much higher rates, over 69%, lasted until October during the increase phase. Very similar patterns were found for males, except that in July 1979 the proportion suddenly dropped to 52% and then remained lower than that of females.

The effects of the three important variables, population density, fluctuation phase, and season, were estimated using the quantification-I method (a multiple regression analysis using dummy variables : Hayashi 1952, Tanaka *et al*. 1984). Population density and fluctuation phase were each divided into four categories, while the seasons were divided into three (Table 1, for internal correlations see Appendix 2). For males, the rate of reproduction was largely explained by population density (partial correlation, 0.732), whereas for females the rate of reproduction was largely explained by the fluctuation phase (0.848). Furthermore, season contributed considerably to the variance of the rate for each sex (0.514 or 0.456), however its partial correlation coefficients were the smallest among the three variables. Thus the intra-population variables affected the reproductive rates of the two sexes in different ways, and made a greater contribution in explaining the reproductive rates than did the climate variable.
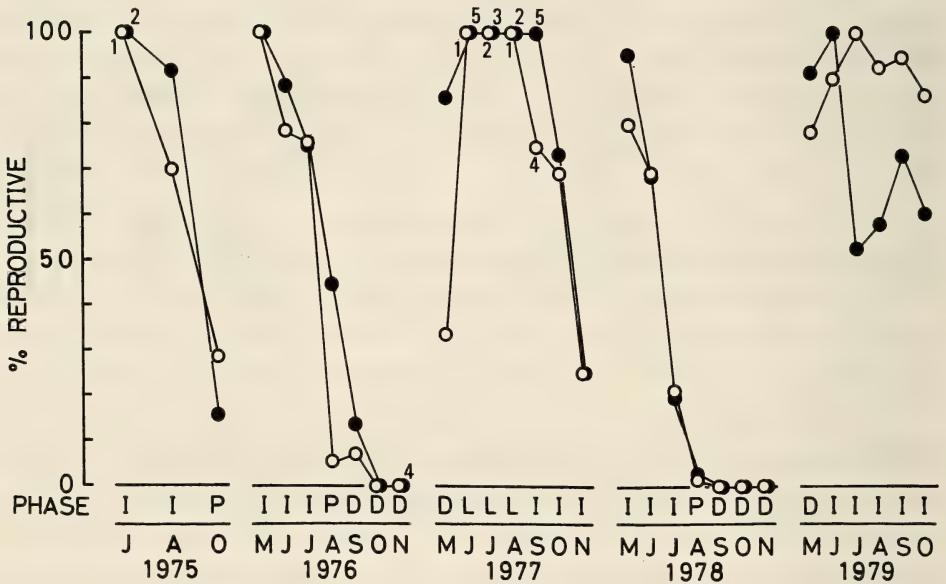
Fig. 3   Changes in the reproductive rate of *A. argenteus*.   ●=males, ○=females.   Numbers are sample sizes of less than six.   Samples from the live-trapping grid and trap-lines were pooled to increase sample size.   Other symbols are as in Fig. 1.

Table 1. Variables and their category scores correlated with reproductive rates of the two sexes in *Apodemus argenteus.*

Variables	Categories	Freq.	Males		Females	
			Scores	Partial cor.*	Scores	Partial cor.*
Population	0–19	10	21.715	0.732	10.631	0.466
density(/ha)	20–39	9	−4.124		−0.965	
	40–59	5	−5.994		−4.390	
	60–79	4	−37.518		−18.920	
Fluctuation	Increase	11	11.508	0.679	6.999	0.848
Phase	Peak	3	−8.171		−28.727	
	Decline	6	−26.822		−39.146	
	Low	8	7.357		30.509	
Season	May-Jun	9	15.246	0.514	12.688	0.456
	Jul-Aug	9	−3.435		−4.827	
	Sep-Nov	10	−10.630		−7.075	
Multiple correlation coefficient (R^2)				0.811		0.838

*Partial correlation coefficient.

Reproductively active mice were trapped until August or September during the decline phase in the high-density years of 1976 and 1978, whereas they were found until October or even November during the increase phase of the low-density years 1977 and 1979 (Fig. 2). In the latter two years, the extended reproductive activity included elements of the following two cohorts. During 1979, for example, the mice that had over-wintered continued to breed until as late as October, and a large number of mice of the year bred between July and October (see also Appendix 1). Similarly, both continuance and participation were found in 1977. In contrast, in the high-density years of 1976 and 1978, over-wintering mice played a large part in reproductive activity by June or July compared with just a few mice of the current year which reproduced by July.

Regarding the relationship between the number of reproductively active mice and the number of potentially reproductive mice (the potential density), the number of reproductively active females increased in proportion to the potential female density at lower density levels, but then decreased at higher potential female densities (Fig. 4). A similar relationship was found for males. The maximum number of reproductively active males in a one-hectare grid was lower than that of reproductively active females: 15 males and 21 females.

Reproductively active mice were found in November 1977, when the mean temperature was 4.0 °C, but not in either October 1976 when the temperature was 10.4 °C, or September 1978, when it was 14.3 °C (ten day means were obtained from meteorological data). Furthermore, reproductively active mice occurred naturally in October 1977 and October 1979, when the mean temperatures were both 11.0 °C. Thus the relationship between autumn temperature and the occurrence of reproductively active mice was contradictory.
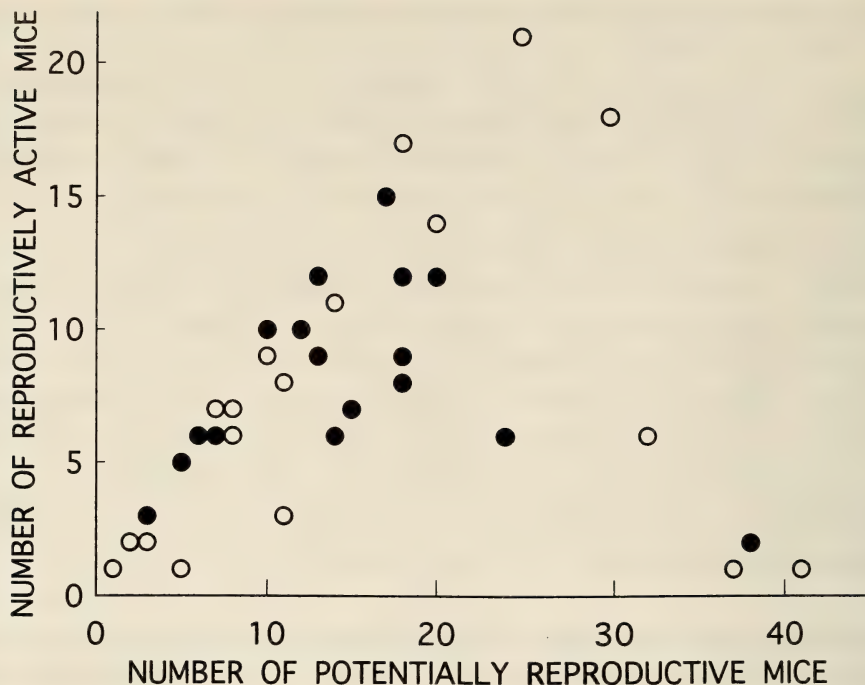
Fig. 4   The relationship between the number of reproductively active individuals and of potentially reproductive individual live-trapped *A. argenteus*.   ●=males, ○=females.

DISCUSSION

Henttonen *et al.* (1985) used an *s*-value greater than 0.5 and a summer decline to classify populations as cyclic.   Furthermore, among *Microtus* populations, Taitt and Krebs (1985) revealed that the amplitude of a cyclic population is usually more than ten-fold. The present study population was found to : have an *s*-value of 0.567 in August samples ; decline during summer ; and have an amplitude of more than 28-fold.   According to Henttonen *et al.*'s (1985) and Taitt and Krebs' (1985) criteria, the fluctuation observed during this study may be regarded as cyclic.

The population fluctuations of *A. argenteus* have been commonly found to be rather stable, repeating similar seasonal patterns from year to year (*e.g.,* Fujimaki 1969, Nishikata 1979). The density variation described in this study substantiates the wider variability of population fluctuation, and essentially provides the first example of cyclicity in a population of this species.

Age and body weight at sexual maturity were closely associated with the fluctuation phase.   Mice matured sexually as early as 30–60 days of age during the increase and low phases (see Appendix 1).   The rapid maturity achieved among the autopsied samples was exactly the same as that under laboratory conditions (Fujimaki and Kuwahata 1985).   In contrast, delayed maturity occurred with greater frequency during the peak and decline phases in the

high-density years. Such changes in maturation closely resemble those of arvicoline rodents (Krebs and Myers 1974, Nakata 1989).

Reproductive intensity was found to be both density- and phase-related (Table 1, Figs. 2, 3 and 4). Considering that decreasing rates of reproduction, and delayed maturation, both occurred earlier in high-density years, these changes are thought to suppress reproductive output and thus accelerate population decline. It suggests, therefore, that the principal regulating factors act on the density- and phase-related reproductive activity. Density-related population regulation has also been described by Montgomery (1989) for *Apodemus sylvaticus*, although Murakami (1974) disregarded the significance of density in population regulation of *A. speciosus*.

The limitation of the number of reproductively active mice may be a key attribute causing reproductive inhibition in a given year. Although a temporal reduction in the proportion of reproductively active adult females has been reported at high densities in some *Apodemus* populations (*e.g.,* Watts 1969, Nishikata 1979, Montgomery 1989), the factors limiting the number of re-productively active mice has hardly been demonstrated so far. Ostfeld (1985, 1990) hypothesized that female numbers are self-regulating through female territoriality, and that female numbers partly or wholly determine male num-bers in relation to mating success. Although the mechanism was not elucidat-ed in our study population, Ostfeld's (1985, 1990) arvicoline-based hypothesis is as plausible for *A. argenteus* as for *A. sylvaticus* (see Wilson et al. 1993). In comparison with the sympatric *Clethrionomys rufocanus*, reproduction of *A. argenteus* was suppressed at lower density levels with smaller observed maxi-mum number of reproductively active individuals for each sex, though the density amplitudes for both species were almost the same (Nakata 1989). Such interspecific differences in reproductive suppression were also observed among mature females of the sympatric *A. agrarius* and *Microtus arvalis* (Bujalska 1981). These findings suggest that reproduction inhibition is more intense in *Apodemus* than in arvicoline species. In other words, reproduction inhibition probably depends on differences in home range size and in socio-spatial organi-zation between mice and voles.

Geographical variation in the timing of the breeding seasons of *A. speciosus* and *A. argenteus* seems to be related more intimately with temperature than with day length (Murakami 1974, Kimura 1977). According to Nishikata (1979), *A. argenteus'* breeding seasons occur in spring when mean temperatures range from 2.5 to 13 °C, and in autumn when they range from 22.5 to 11.5 °C. In our study population, however, reproduction occurred in autumn 1979 at tem-peratures well below 11.5 °C and contrarily reproduction did not occur above 11.5 °C in autumn 1978 (Fig. 5). Thus temperature does not always cause the variations in the breeding season of wild *A. argenteus*.

When a population fluctuates in a uniform pattern from year to year, the effect of climate on the breeding season is likely to be well documented. In contrast, when a population exhibits significant multi-annual fluctuations, the effects of ecological factors such as density, seed yield and/or predation are

thought likely to be of importance relative to the proximate factors.   Adamcz-
ewska (1961) found that an *A. flavicollis* population reproduced either in
autumn or in spring-summer, and assumed that the crop of tree seeds exerted
an important influence on the duration of the breeding season.   Jensen (1982)
then found that *A. flavicollis* extended its breeding season into winter in years
when trees produced large crops of seeds.   Furthermore, *A. semotus'* summer
decline in breeding, described by Lin and Shiraishi (1992), is a consequence of
large numbers of yearlings entering the population.   These findings also
discredit the effect of temperature on breeding by *Apodemus* spp.
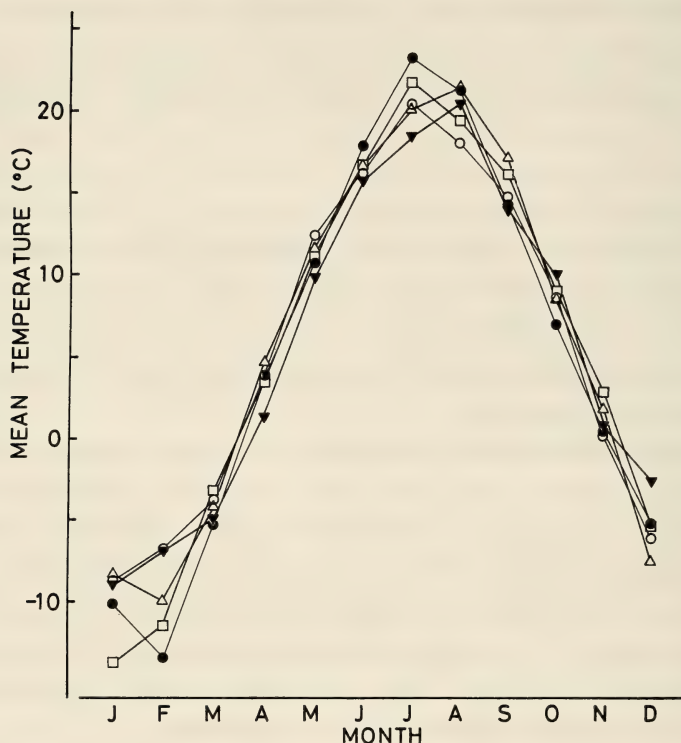
Fig. 5   Monthly mean temperature records at Higashikawa Meteorological Station.   △=
1975, ○=1976, □=1977, ●=1978, ▼=1979.

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Appendix 1.   Age distribution of trapped *A. argenteus*.   Each small rectangle represents one autopsied mouse.   ■ = reproductive, ▨ = post-reproductive, □ = immature mouse.   Age classes were :   I = 25 days old, II = 1-2 months old, III = 2-4 months old, IV = 4-10 months old, and V = 10-18 months old.

Appendix 2.   Simple correlation coefficients between reproductive rate (Y), population density(X_1), fluctuation phase(X_2), and season(X_3)

	Males			Females		
	X_1	X_2	X_3	X_1	X_2	X_3
Y	0.746	0.562	0.618	0.637	0.822	0.410
X_1		0.184	0.467		0.386	0.458
X_2			0.178			0.083

Den site selection and utilization by the red fox in Hokkaido, Japan

Kohji URAGUCHI[1] and Kenichi TAKAHASHI[2]

Hokkaido Institute of Public Health, Sapporo 060-0819, Japan
Fax. +81-11-736-9476, [1]e-mail. ura@iph.pref.hokkaido.jp, [2]e-mail. takaken@iph.pref.hokkaido.jp

Abstract.   Den site selection and den use by the red fox, *Vulpes vulpes*, were studied on the Nemuro Peninsula, eastern Hokkaido, Japan.   Certain physical variables of 144 fox den sites were compared with those of 236 randomly selected control locations. The red foxes on the Nemuro Peninsula clearly preferred to den on slopes in woodlands near open spaces and streams.   The seasonal pattern of den utilization was studied from June 1986 to May 1987. Red foxes used dens mainly during the period from January to June.   Since this period coincides with the gestation, parturition and cub rearing periods of the red fox, it was confirmed that the red fox's den was fundamentally a breeding site.   Almost all dens were observed each spring from 1986 to 1996 to establish whether they were used for breeding or not, and it was found that the number of fox families was stable during this decade.

Key words : den, Hokkaido, red fox, habitat selection, *Vulpes vulpes*.

Habitat selection is a reflection of a species' environmental, ecological and physiological requirements.   For the red fox, *Vulpes vulpes*, den sites are very important because the cubs are born there and because they are reared there while still juveniles.   Therefore, foxes might be expected to exercise some preference when choosing locations for their dens.   Although many studies have described fox den characteristics in different habitats (*e.g.,* Scott and Selko 1939, Storm *et al*. 1976, Roman 1984, Zhou *et al*. 1995), there have been few quantitative studies on den site selection by red foxes (Nakazono and Ono 1987, Meia and Weber 1992).

Information on den site preferences, and on the number of breeding dens being used in a given area, are useful for understanding both the habitat evaluation being made by red foxes for reproduction, and any trends in their population dynamics.   Furthermore, such an understanding is helpful in the development of control measures against zoonoses transmitted by red foxes.

In this study, we describe the habitat factors associated with dens that we detected by comparing certain variables from den sites with those of control sites, and we also describe the utilization pattern of fox dens in Hokkaido, Japan.

STUDY AREA

The study area (73.0 km²) is located in the central part of the Nemuro Peninsula in eastern Hokkaido.   The area is composed of low rolling hills with about twenty streams in small eroded valleys with steep slopes.   The highest point was only 55 m above sea level.   The study area consisted of a mosaic of pastures (43.6%), grasslands (24.7%), woodlands (20.7%) and residential areas (11.0%).   The pastures consisted largely of *Phleum pratense* which was cultivated for pasturage and hay-making.   The grasslands were dominated by *Sasa nipponica*, Gramineae spp. and *Artemisia montana*.   The woodlands were principally located along the banks of streams and were dominated by broad-leaved deciduous trees such as *Quercus nipponica*, *Alnus hirsuta* and *Betula ermanii*.   There were also small woodlots of *Abies sachalinensis*.   The climate of this area is cool : the mean February temperature is −5.3°C and the mean August temperature is 17.1°C.   It usually snows from late December to March, and the average yearly precipitation is 1,035 mm (National Astronomical Observatory 1996).   The human population of this area was about 30,000, 95% of whom lived in two residential areas.   There were some fishing ports along the sea coast, and 54 dairy farms were scattered through the area.

METHODS

The field work for this study was conducted from 1986 to 1996, however forty-eight fox dens had already been located before the main field study began as a result of questioning farmers and from field inspections made during 1984 and 1985 (Kondo pers. comm.).   Since this preliminary information suggested that there were few dens in pastures, we searched for fox dens in grasslands and woodlands mainly during May and June 1986.   Because most of the woodlands in this area were located along streams, almost all stream banks were inspected.   Stream banks were usually surveyed by one observer traversing upstream along one bank and downstream along the other.   In open areas, such as grassland slopes, binoculars were also used.

Most dens observed in Hokkaido consist of tunnels with a diameter of some 20 cm excavated by the foxes themselves.   Although rabbits, *Oryctolagus cuniculus*, and badgers, *Meles meles*, dig tunnels in this size range elsewhere, hence leading to some difficulties of identification (Cowan 1991, Roper 1992), neither rabbits nor badgers are found in Hokkaido.   Raccoon dogs, *Nyctereutes procyonoides*, possibly use such tunnels as their dens, but few individuals occur in this study area (Kondo pers. comm.).   Therefore, we regarded all excavated tunnels with a diameter of circa 20 cm as fox dens.

All dens were marked on a 1 : 50,000 map, and numbered in the order that they were found.   At each site, we measured a series of variables which were considered likely to be associated with dens, as in previous studies (Zhou *et al*. 1995, Scott and Selko 1939, Roman 1984, Nakazono and Ono 1987, Meia and

Weber 1992). These variables included: 1) habitat type within 10 m of the primary entrance, 2) the number of entrances, 3) eight grade directions of the slope of the primary entrance, 4) the angle of the slope on which the primary entrance was located, 5) the distance to the nearest open space (non-wooded area which was more than 10 m in diameter), 6) the distance to the nearest source of water, 7) the distance to the nearest dwelling house, and 8) the distance to the nearest road.

The occurrence of red fox dens within habitats versus the relative availability of habitats, determined from vegetation maps was tested using the *G*-test for goodness of fit (Sokal and Rohlf 1981). On the Nemuro Peninsula, even if foxes were to excavate dens in pastures, they would soon be destroyed, because the pastures are harvested by tractor every summer and autumn, and are plowed every three to five years. Two fox families that made their dens in residential areas during this study were immediately turned out or captured by city officers as pests. We, therefore, regarded pastures and residential areas as unsuitable habitat for denning by red foxes, and have excluded them from further discussion of den site selection by foxes on the Nemuro Peninsula.

To ascertain which habitat factors influenced den site selection by red foxes, variables from den sites were compared with control sites within grasslands and woodlands. Five hundred control locations were marked randomly on a 1 : 25,000 map ; the 264 control sites that fell within pastures or residential areas were excluded from the analysis leaving 236 control sites within grasslands and woodlands. Distances to the nearest house and road were measured from maps, and the distance to the nearest open space was measured from aerial photographs. Because the direction and angle of a slope and the distance to the nearest source of water were difficult to measure from either maps or aerial photographs, 80 of the 236 control sites were chosen randomly and visited using a hand-held GPS receiver (GPS45, GARMIN INTERNATIONAL) and the variables of the sites were measured directly.

The habitat surrounding fox dens and habitat availability were compared using a 4×2 *G*-test of fitness. The mean values of the angle of the den slope and four kinds of distances for both den sites and control locations were compared using the two-tailed Mann-Whitney *U*-test. The frequencies of eight grade directions of the slope in the two samples were compared using an 8×2 *G*-test of independence (Sokal and Rohlf 1981). Repeating individual statistical tests increased the chance of type I errors. To compensate for this, we took the standard probability of $p \leq 0.05$ and divided it by the total number of tests ($n = 6$) looking for differences in physical variables between den sites and control locations (Ortega 1987). Consequently, the conservative significance level ($p \leq 0.008$) was used.

Dens were defined as either a) unoccupied, b) occupied but without cubs, or c) occupied with cubs (breeding dens) based on the presence of signs found during monthly visits from June 1986 to May 1987. The distinction between dens with or without cubs was based either on the direct observation of cubs, or on the presence or absence of conspicuous marks indicating their presence,

i.e., polishing of excavated soil by cubs moving in the out of the den, fecal remains, and signs of play such as flatten grasses.

From 1988 to 1996, dens were usually observed just once a year in spring in order to check the breeding status of the fox population. As the peak of fox parturition occurs from late March to late April in Hokkaido (Abe 1974), and because juveniles usually begin to emerge from the dens when about six weeks old, that is during May (Lloyd 1980), we mainly observed dens during the latter half of May.

Red foxes are susceptible to even slight disturbance, and often move their juveniles from one den to another (Sargeant 1975, Storm *et al.* 1976, Lloyd 1980, Stubbe 1980, Nakazono and Ono 1987) making it difficult, therefore, to distinguish between natal dens and to which juveniles have been moved (rearing dens). In this paper, therefore, we have used the term "breeding den" to include both natal and rearing dens. Given the risk of disturbing the foxes and causing them to move by observing them, adjacent dens were always observed on the same day so as to avoid double-counting litters.

A single vixen and her cubs might use several breeding dens, hence the number of breeding dens used did not equate to the number of families. In this study, the minimum distance from a breeding den to an adjacent family was assumed to be 500 m because 12 out of 15 known den translocations involved movements of less than 500 m from the original den as indicated by radio-tracking and tag observation (Uraguchi unpublished). Dens within 500 m of each other were regarded, therefore, as belonging to one family, and all other dens were assigned to different families.

RESULTS

1. Den site selection by red fox

A total of 161 fox dens were found in the study area by May 1996. The defining variables of 144 of those dens were recorded (the remainder were either destroyed by man or collapsed naturally). One hundred and twenty-eight, out of the 144 dens (88.9%), consisted of tunnels excavated by the foxes themselves, while the remaining 16 dens were artificial (underfloors of abandoned houses or warehouses, and under concrete debris). One den was found in pasture land, although systematic searching was not conducted in this habitat.

Table 1. A comparison between the habitats of red fox den sites and habitat availability in a study area on the Nemuro Peninsula ($n=140$).

	Habitat type			
	Deciduous forest	Coniferous forest	Mixed forest	Grassland
Percent available	40.0	4.5	1.0	54.5
Observed number (%) of dens*	77 (55.0)	5 (3.6)	5 (3.6)	53 (37.9)
Expected number of dens	56.0	6.3	1.4	76.3

*Excepted four dens that were situated pastures or under the floors of houses.
$G=20.8$, $d.f.=3$, $p<0.005$.

Table 2.　Mean values (±SD) of physical variables of fox den sites and control sites within woodlands and grasslands on the Nemuro Peninsula.

Variables	Den sites	n	Control sites	n	p
Angle of slope (°)	32.5 (±15.2)	107	15.9 (±14.4)	80	<0.0001*
Nearest stream (m)	85.6 (±134.9)	144	185.9 (±260.0)	80	<0.0001*
Nearest open space (m)**	18.7 (±20.7)	87	33.4 (±35.0)	85	0.0003*
Nearest house (m)	408.7 (±248.6)	144	366.3 (±271.8)	236	0.0251
Nearest road (m)	276.1 (±212.8)	144	283.8 (±217.5)	236	0.8517

*$p \leq 0.008$
**Comparison between den site and control site within forests.

　　Red fox den sites were not distributed randomly according to habitat availability. Dens were found more often than expected in woodlands and less often than expected in grasslands (Table 1). Furthermore, dens within woodlands were located significantly closer to open spaces than were control locations within woodlands ($p=0.0003$, Table 2). In our study area, most of the open spaces close to dens were grasslands. Fox dens were also located significantly closer to water sources (usually a stream), and on steeper slopes than the control sites. Den sites and control locations did not differ significantly in their distance from either the nearest house or the nearest road. The direction that slopes on which dens were located faced were recorded at 111 den sites and at 69 control locations. Dens occurred more frequently on slopes facing west and south-west and less often on slopes facing east or south-east than control locations, but this difference was not significant ($G=15.4$, $d.f.=7$, $p=0.03$). The average number of entrances per den for 140 dens was 3.5 ± 3.6 (mean$\pm SD$, range 1–36).

　　Five physical variables of 20 dens used for breeding more than five times during the 11 year study were compared with those of 21 dens that were never used for breeding during the same 11 years. There was surprisingly no significant difference between them (Table 3).

2. Seasonal patterns of den utilization

　　Although fox dens were utilized all year around, the proportion of dens utilized varies seasonally (Fig. 1). The percentage of dens utilized decreased during July, and remained low until December, then increased again during January, and remained high until June. Dens occupied by cubs were found from April onwards, but most of them were observed during May, June and July.

3. Annual change in the number of breeding dens

　　The numbers of breeding dens and the numbers of families (estimated by the use of the 500 m criterion) were calculated each year from 1987 to 1996, though not from 1986 because the sample size that year was too small (Table 4). The number of the breeding dens was 22–41 and the estimated number of families was 20–31. There were no significant differences between successive

Table 3. Mean values (±*SD*) of physical variables at dens used for breeding more than five times (*n*=20) and dens never used for breeding (*n*=21) during the 11 years from 1986 to 1996 on the Nemuro Peninsula.

Variables	Breeding dens	Non-breeding dens	*p*
Angle of slope* (°)	36.3 (±11.0)	29.6 (±15.4)	0.402
Nearest stream (m)	85.0 (±148.7)	114.9 (±178.0)	0.657
Nearest open space (m)	16.3 (±19.5)	15.5 (±23.5)	0.408
Nearest house (m)	406.3 (±175.3)	419.3 (±222.2)	0.927
Nearest road (m)	248.8 (±148.1)	238.9 (±185.9)	0.676

*For this variable only, the sample size for breeding dens was 19, and that for non-breeding dens was 18.

years in either the numbers of breeding dens used or the estimated number of families, however, the number of breeding dens fluctuated more than the estimated number of families, and their trends were not always consistent with each other.

Eighty-two dens were observed every year from 1986 to 1996. Of these 82, 61 (74.4%) were used for breeding at least once during the 11 year study. One den was used 11 times, three were used nine times each, and six were used eight times for breeding. These estimates are considered to be lower than actuality, because most dens were visited only once a year from 1988 onwards, thus breeding activity may have been missed.
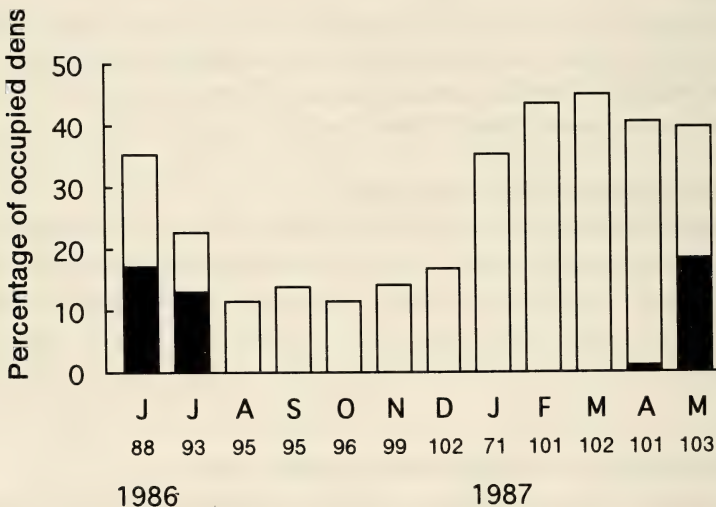
Fig. 1. Monthly variation in the proportion of dens occupied during the period from June 1986 to May 1987. ■: dens with cubs, □: dens without cubs. The number below each column represents the number of dens observed.

Table 4. The number of breeding dens and estimated families. Expected numbers were calculated from the ratio of the average number of breeding dens and families to the average number of observed dens.

Year	No. of observed dens	No. of breeding dens	Expected no. of breeding dens	No. of families	Expected no. of families
1987	106	27	28.9	20	21.8
1988	104	32	28.4	25	21.4
1989	113	36	30.9	22	23.2
1990	114	34	31.1	22	23.4
1991	114	29	31.1	22	23.4
1992	118	22	32.2	21	24.3
1993	117	41	31.9	29	24.1
1994	122	32	33.3	25	25.1
1995	133	27	36.3	24	27.3
1996	131	40	35.8	31	26.9
Average	117.2	32.0 (27.3%)		24.1 (20.6%)	
	$d.f.=9$	$G=10.8, p>0.1$		$G=3.4, p>0.9$	

DISCUSSION

Although red foxes are able to make their dens in various environments such as in woodlands, grasslands, plowed fields, pastures, dunes, among rocks and residential areas (Sheldon 1950, Nakazono 1970, Sargeant 1972, Abe 1974, Storm *et al.* 1976, Harris 1977, 1981, Macdonald and Newdick 1982, Roman 1984, Nakazono and Ono 1987), in this study area, their dens were strongly associated with relatively steep slopes near streams and open spaces in woodlands. The question remains open as to why they prefer these areas rather than others for denning.

Den sites on steep slopes, as found during our study, may well be advantageous because of their good drainage. Some previous studies have also demonstrated that many fox dens are to be found in well-drained soil (*e.g.,* Scott and Selko 1939, Sheldon 1950, Roman 1984), and most dens have been found on slopes with gradients of 5–10% or more (Scott and Selko 1939). On the Nemuro Peninsula, the angle of the slopes on which primary den entrances were located were relatively steep (mean$\pm SD=32.5\pm15.2°$). Such den site selection may have been related to the fact that the soil of the study area consisted largely of Gleyic Cumulic Andosols which are badly drained (Hokkaido National Agricultural Experiment Station 1985). Slopes may be advantageous for denning for other reasons in addition to drainage. Digging and the removal of soil may be easier, for example, and on steep slopes perhaps rain and snow are less likely to fall into the dens.

Goszczyński (1989) described forests as primary shelter for foxes and for raising their young. Woods may also serve to provide shelter for juvenile foxes. In the present study area, however, fox dens within woodlands were situated closer to open spaces than were control sites within woodlands indicat-

ing that open areas are also important for them. Nakazono and Ono (1987) suggested that juvenile foxes require substantial amounts of sunshine for normal growth. Marginal sites in woodlands might be preferable both for sheltering and for providing sunning opportunities for juveniles.

Although many fox dens were situated near streams, we do not believe them to be essential for drink water, because adult foxes are able to find water to drink in many situations. Moreover since one den, located 300 m away from a stream, was used for breeding four times during five years, a stream does not seem necessary for juveniles to drink at either. The Nemuro Peninsula experiences many foggy days during late May and June when young foxes are being raised in the breeding dens. It is more than likely that the cubs are able to obtain sufficient water by licking leaves wet with fog and from the food provided by their parents. There is a tendency for steeper locations to be closer to a source of water (Fig. 2), thus the reason why many dens were situated near streams was probably the result of the foxes' preference for well-drained, steeper slopes.

No difference was found in five physical variables between breeding and non-breeding dens. During the course of this study, we were unable to evaluate the impact of disturbance by other animals, especially humans and stray dogs, on fox breeding, because it was difficult to express quantitatively. Disturbance is, however, considered an important variable affecting selection and
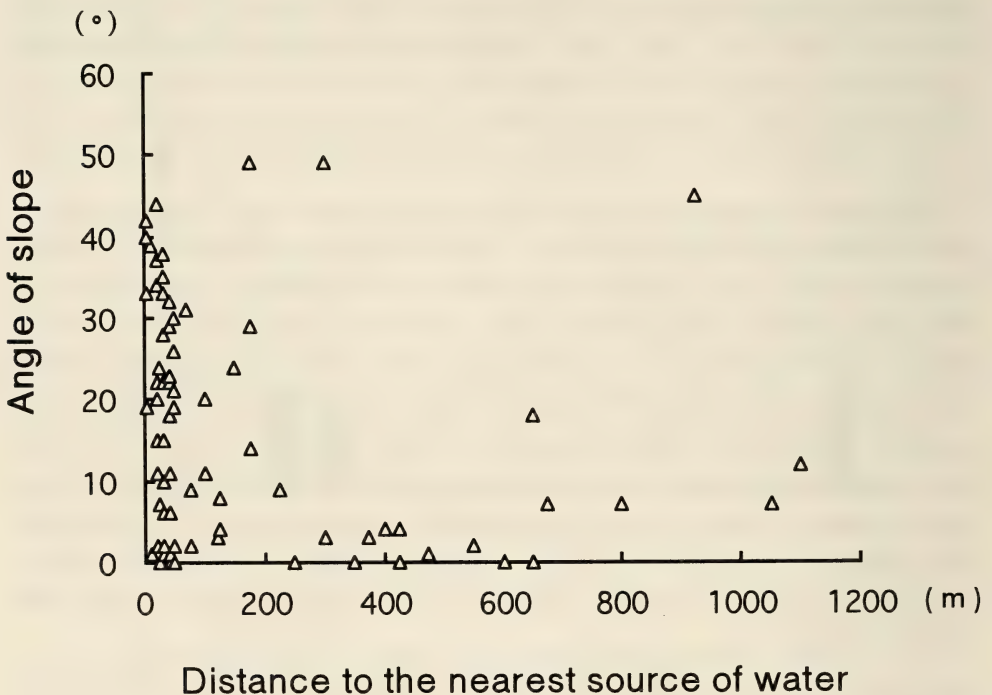
Fig. 2. The relationship between the angle of slope and the distance to the nearest water source of 80 control locations.

utilization of fox den sites (Storm *et al.* 1976, Harris 1977, 1981). One reason why no difference was found between breeding and non-breeding dens might be because of the absence of any measure of this "disturbance" factor in our analysis.

In the Nemuro area, fox dens were mainly used during the period from mid winter to early summer, a tendency also reported for the red fox in Kyushu, southern Japan (Nakazono and Ono 1987). In Hokkaido, fox mating peaks from late January to mid February followed by a peak in parturition from late March to late April (Abe 1974). The period during which dens were used most intensively in the Nemuro area corresponded, not surprisingly, with the period of mating, parturition and rearing of cubs, confirming that dens are fundamentally breeding sites for the red fox. Of interest, therefore, is the fact that about half of the dens occupied were not used for rearing cubs during the later winter and early spring period, and 11-17% of dens were used even during the period from August to December, though not for breeding. Since few of the signs typical of frequent use such as polishing of excavated soil were observed, these dens might have served just as temporary retreats (Nakazono and Ono 1987).

The density of families estimated using the 500 m criterion was 0.27-0.42 families/km², and was stable over a period of 10 years. There have been few studies on the density of fox families in Japan, however, the density in Nemuro was clearly higher than in either Yabe, Kyushu (0.18 families/km², Nakazono and Ono 1987) or in Koshimizu, Hokkaido (0.24 families/km², Abe 1974). Some studies in southern Sweden, central Europe and England have indicated that where vole densities are high, then fox populations became socially regulated stable and dense. The relatively high and stable density of fox families on the Nemuro Peninsula is probably due to the high density of voles in the area (Saitoh and Takahashi 1998) and the richness of alternative food, such as organic waste from fisheries and from dairy farms (Kondo *et al.* 1986).

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Improvement of errors in radiotelemetry locations of brown bears, *Ursus arctos*, in Hokkaido, Japan

Takahiro MURAKAMI[1] and Tsutomu MANO[2]

[1]*Laboratory of Ecology, Department of Environmental Veterinary Science, Graduate School of Hokkaido University, North 18 West 9, Kita-ku, Sapporo 060-0818, Japan*
Fax. +81-11-706-5569, e-mail. ursus@vetmed.hokudai.ac.jp
[2]*Wildlife Section, Nature Conservation Department, Hokkaido Institute of Environmental Sciences, North 19 West 12, Kita-ku Sapporo 060-0819, Japan*

Abstract. The errors and the sources of errors made while fixing radiotelemetry locations were estimated in two brown bear, *Ursus arctos yesoensis*, study areas, in the Shiretoko and Oshima regions of Hokkaido. We measured the sampling error and the bias of test transmitters placed at various points in our study areas. The means of our sampling errors were approximately equal to those previously reported by Springer (1979), while the maxima of our sampling errors and the means of our biases were larger than those of Springer's (1979). We also assessed the amount of error in estimating locations based on measuring three bearings. As the sizes of triangles were positively related to the degree of error in estimating their points, we excluded large (>6.25 ha) triangles from the analysis. The maximum values of the 99% confidence intervals for normalized error distances were 321.4 m in the Shiretoko area and 302.3 m in the Oshima area. These values were compared with Lenth's (1981) Andrews estimators calculated from the original data sets of both study areas. The direct method for estimating radiotelemetry error which we used in this study is easy to calculate and proved not to be inferior to Lenth's (1981) method.

Key words: brown bear, error estimation, field test, radiotelemetry, triangle size.

Radiotelemetry is often used to locate free-ranging wild animals. Numerous studies including those of home range, habitat use and movement have depended on radiotelemetry (Samuel and Fuller 1994). Locations, which are estimated by radiotelemetry do, however, include a certain degree of error (Springer 1979, Zimmerman 1990).

Because brown bears have much larger home ranges than most other terrestrial mammals, and because they are difficult animals to approach, the estimated locations of radio-collared bears may contain large degrees of error. Saltz (1994) indicated that many radiotelemetry studies did not describe their degree of error. Saltz (1994) further asserted that researchers should both measure and describe their degree of error on the basis that if a radiotelemetry study were conducted without error estimation, then it could lead to misunder-

standings of animal movements or of their habitat utilization patterns.

Various methods of estimating location error have been described (Heezen and Tester 1967, Springer 1979, Lenth 1981, Garrott *et al*. 1986), however, Zimmerman and Powell (1995) considered that these methods, over-estimate location errors when applied to field data. Furthermore, some of these methods have strict prerequisites and some require long calculation processes.

In Japan, Maruyama *et al*. (1978) measured the distances between the estimated and the actual locations of four Sika deer, *Cervus, nippon* fitted with transmitters. Maruyama *et al'*s (1978) study, however, described only the extent of error under special conditions, and neither indicated how to detect or decrease errors. Since Maruyama *et al'*s (1978) study, radiotelemetry studies reported in Japan have paid little or no attention to error. Improvement of the error estimation method in the field and the promotion of a greater awareness of it among researchers is necessary for the appropriate interpretation of large mammal behavior.

Zimmerman and Powell (1995) introduced an original error indicator which was based on the statistics derived from the linear distance between the actual and the estimated locations of test transmitters. We also used test transmitters in the field in order to estimate the degree of error of the location method, a method often used in Japan, and compared it with Lenth's (1981) Andrews estimator. By using our method, researchers who use radiotelemetry to study large mammals can easily estimate the area of error of their estimated locations and can improve the precision of their estimation.

Study Area

Our study was conducted in two brown bear radiotelemetry study areas, one of which was in the Shiretoko National Park in eastern Hokkaido, and the other of which was on the south-western part of the Oshima Peninsula in southwestern Hokkaido, Japan.

In the first of these two areas, the Shiretoko-Renzan mountains, ranging in height from 700 m to 1,600 m, run along the center line of the Shiretoko Peninsula. The flanks of these mountains meet the coastline abruptly. Japanese stone pine, *Pinus pumila*, is dominant above 500–600 m, while at lower elevations mixed forest predominates. There are two paved roads which facilitate radio tracking inside the study area where the road density is 0.37 km/km².

The second study area, the south-western part of the Oshima Peninsula, is also mostly mountainous, but here the mountains range from just 200 to 600 m in altitude. The terrain is more rugged than in the Shiretoko study area because there are many steep streams. The most common vegetation here is deciduous forest. There are two paved roads and several forestry tracks inside the area which facilitate radio tracking. The road density here is 0.44 km/km².

MATERIALS AND METHODS

1. Measurement of Bias and of Sampling Errors

Radiotelemetry error is derived from a combination of bias and sampling error (Springer 1979), where bias is the angle between the measured value and the true direction of the transmitter, and where sampling error is the amount of variation in estimated values when repeatedly taking bearings off the same transmitter and when using the same apparatus. In order to quantify such bias and error, we set several 144-147 MHz radio transmitters (Telonics, Inc., Mesa, Arizona, or Loteck, Inc., Aurora, Ontario) within the study area. Someone who was unaware of the transmitter's location was selected to measure sampling errors and biases from several points on one of the roads in the study area.

Directions were determined using a 3-element Yagi-antenna and an FT290-mk II receiver (Yaesu Musen, Inc., Tokyo). The direction from each receiving point to each transmitter was measured 10 times. Following Springer (1979), we considered pooled standard deviations as the sampling error, and regarded the angles between the average bearings of measured values and the true directions as the biases. After one set of measurements was made, the transmitter was moved to another location, and the procedure was repeated. We included biases derived from the following tests in our analyses.

A map of the study area was overlaid with a grid of 500 m square quadrats. Because topographical similarity affects results, it was decided to use only one transmitter set point within a single quadrat. Transmitters were set at 21 points in the Shiretoko study area, and at eight points in the Oshima area. The distances between the observers and the transmitters, ranged from 350 m to 2,150 m ($m \pm SD = 1,156.2 \pm 569.9$ m) in the Shiretoko study area, and from 175 m to 3,750 m ($m \pm SD = 1,770.6 \pm 951.2$ m) in the Oshima area.

2. Measurement of the Error of Estimated Locations

Maruyama *et al.* (1978) described an original method which considered the centroid of the triangle, derived from three bearings as the location of the transmitter. Some researchers in Japan have used this method (Hokkaido Institute of Environmental Sciences. 1995), which we now call the Triangle Center Method or TCM.

Transmitters were set at various points in the study areas, as described in the previous section; 17 transmitter set points were used in Shiretoko and 30 in Oshima. We measured the bearings of the signals from three to nine receiving points for each transmitter set point and recorded each bearing and receiving point on 1 : 25000 scale topographical maps. The location of each transmitter set point was calculated using the TCM by a researcher who did not know its location. We then gauged the distances between the true locations and the centers of triangles derived from any three bearings. Large triangles exceeding 6.25 ha, which is the size we use in our brown bear study, were excluded

from location estimation in order to improve reliability. This method was used after confirmation of the positive correlation between triangle size and error distances.

Lenth's (1981) Andrews estimator was considered by White and Garrott (1990) to be the most reliable way to estimate location errors. We calculated Andrews estimator from our data (computed by TRIANG [Garrott *et al.* 1986], provided by G. C. White), and compared this with the TCM error.

The distances between observers and transmitters in our study ranged from 75 m to 4,063 m ($m \pm SD = 1,392 \pm 769.2$ m) in the Shiretoko area, and from 100 m to 3,750 m ($m \pm SD = 1,185 \pm 769.0$ m) in Oshima. These values differed from those obtained in the previous section, because the transmitter set points were different from those used when measuring bias and sampling error.

RESULTS

1. Bias and Sampling Error

Mean sampling errors were 4.3 ± 1.7 (*SD*)° in Shiretoko area, and 6.0 ± 4.5 (*SD*)° in Oshima area. The maximum sampling errors recorded were 8.7° in Shiretoko area and 20.2° in Oshima area. Biases ranged from $-172°$ to $+61.5°$ in Shiretoko area, and from $-108°$ to $+140°$ in Oshima area. Biases averaged -2.3 ± 27.7 (*SD*)° in Shiretoko area, and -3.6 ± 31.9 (*SD*)° in Oshima area (see Fig. 1 for the frequency distribution of biases in each study area).

2. Error of Estimated Location

In Shiretoko area, 188 triangles were derived from triangulation of 17 transmitter set points. In Oshima area, we obtained 133 triangles from 30 transmitter set points. The means of estimation errors were 495.5 ± 467.4 (*SD*)
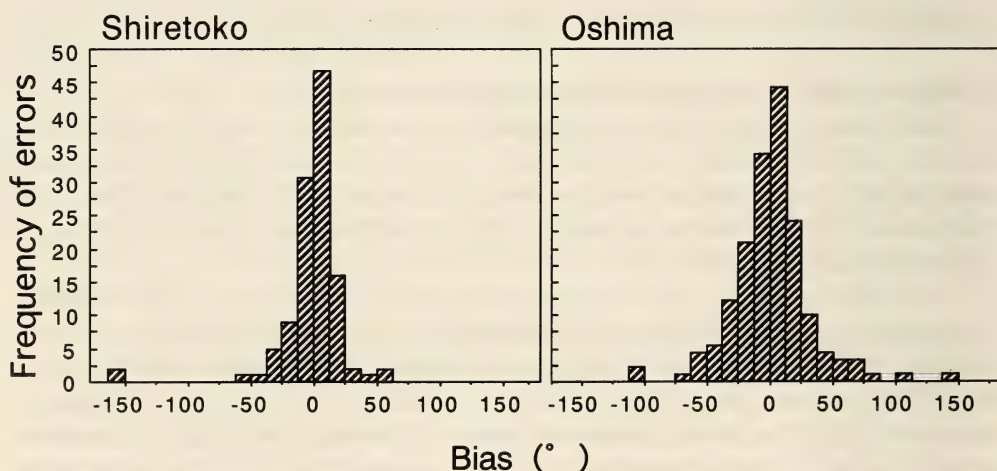
Fig. 1. Frequency distributions of biases accompanied by bearings in the Shiretoko and Oshima study areas. Biases ranged from $-172°$ to $+61.5°$ in Shiretoko area and from $-108°$ to $+140°$ in Oshima area.

m in Shiretoko area and 339.2±286.3 (*SD*) m in Oshima area.

There was a positive correlation between triangle size and estimated location error when using the TCM (Fig. 2, Kendall's $\tau=0.23$, P<0.01 in Shiretoko area and $\tau=0.35$, P<0.01 in Oshima area). When we excluded large triangles (those exceeding 6.25 ha) from the analysis of location error, we obtained a frequency distribution of location errors (Fig. 3). As these distributions were not parametric (Shapiro-wilk test for normality, P<0.01), we calculated the cube root of each value to obtain a normal distribution. After transformation, the means of the TCM errors were 260.6±7.28 (*SD*) m in
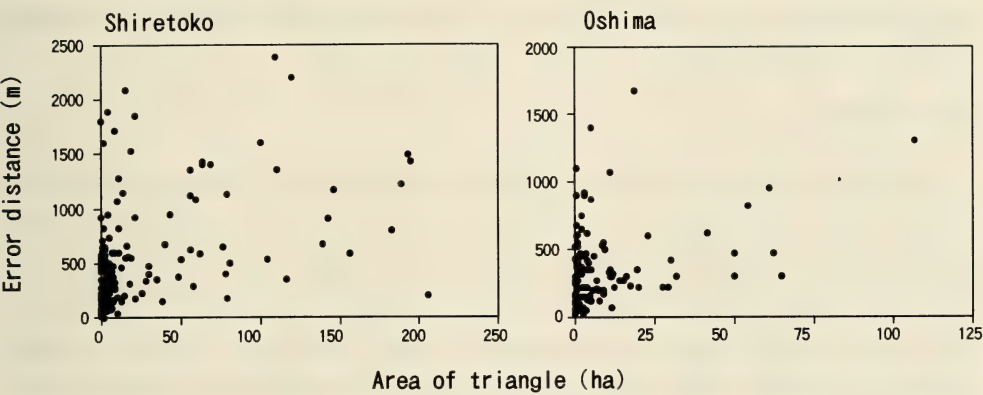
Fig. 2. The relationships between the estimated location error of the TCM and the areas of triangles derived from three measured bearings. Estimated locations from larger triangles tend to have larger location errors.
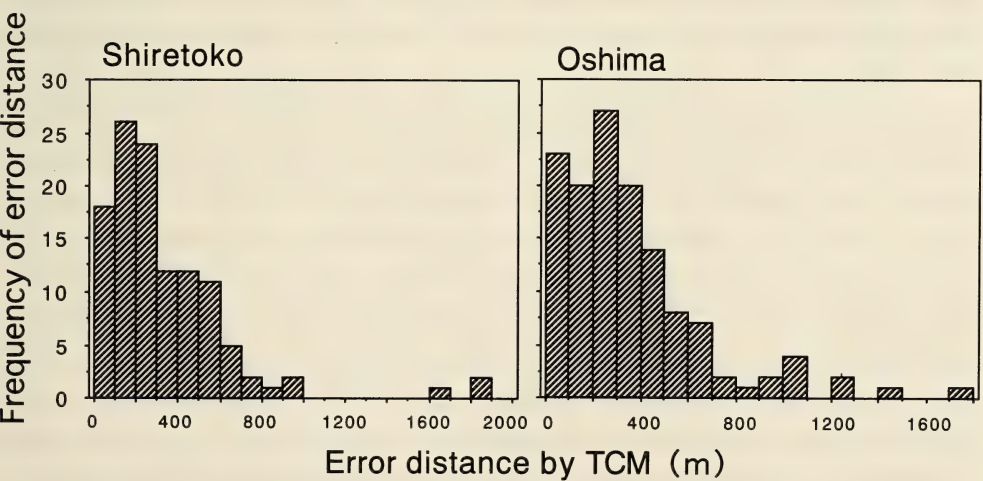
Fig. 3. Frequency distributions of error distances between locations estimated by TCM and true locations.

Shiretoko area and 241.7±5.96 (*SD*) m in Oshima area.   The maxima of the 99 % confidence intervals were 321.4 m in Shiretoko area and 302.3 m in Oshima area.

In the same way (though using natural logarithms instead of cube roots for normalization of the Oshima data sets), the means of the location errors found by Andrews estimator for the same data sets were calculated to be 258.5±61.08 (*SD*) m in Shiretoko, and 297.7±7.53 (*SD*) m in Oshima.   The maxima of the 99% confidence intervals in Shiretoko were 319.9 m, and 381.6 m in Oshima. The means of TCM errors did not differ significantly from those of Andrews estimator in Shiretoko (Student's *t*-test, $t = 0.37$, $p > 0.05$), while in Oshima, the mean of Andrews estimators was significantly larger than that of TCM errors (Student's *t*-test, $t = 54.3$, $p < 0.01$).

DISCUSSION

Springer (1979) who measured biases and sampling errors in field trials reported that sampling errors ranged from 3.4° to 4.3° and biases ranged from −0.4° to 1.7°.   In our field tests, although the means of the sampling errors (4.3° and 6.0°) were nearly equal to Springer's (1979) values, the maxima (8.7° and 20.2°) were much larger.   The means of our biases, −2.3° and −3.6°, did not differ significantly from zero (Student's *t*-test, Shiretoko : $t = 0.63$, $p > 0.05$; Oshima : $t = 1.00$, $p > 0.05$), however the ranges of our biases were much wider. These differences may be accounted for by differences in study conditions. Springer's (1979) study area was comparatively flat, whereas our study area contained steep terrain.   Sometimes we received radio signals from a very wide range of directions and as a result we experienced large sampling errors. Hilly terrain also generates large biases (Lee *et al*. 1985).   Thus, when using radiotelemetry in mountainous areas such as the Shiretoko and Oshima Peninsulas, we must take into consideration the likelihood of large sampling errors and biases.

Large biases and large sampling errors cause large location errors.   It appears that estimated TCM locations may include large errors.   Maruyama *et al*. (1978) reported a TCM error of 123±11.8 (*SD*) m on Kinkazan Island, however, the maxima of the 99% confidence intervals of the TCM errors for our two study areas were even larger at over 300 m.   We consider that this difference may have been caused by differences in our experimental methods. Maruyama *et al*. (1978) did not describe the distance between their transmitters and receivers, which, on the basis of the figures that appear in their report, may have been under 800 m.   In contrast, the mean distances between transmitters and receivers in our study was greater than 1,000 m.   Furthermore, the topography of Kinkazan Island is less rugged than that of either of our study areas. These factors, we believe, may well have affected the results.   Zimmerman and Powell (1995) considered the arithmetic mean of the compass bearing intersections, derived from three bearings, to be the estimated location, and reported the mean of the linear distances between their estimated and true locations as

279 m. Our TCM error was very similar to this value, and our study conditions were also similar to theirs. Their tracking distances ranged from 300 m to 6,020 m, while ours, ranged from 75 m to 4,063 m and from 100 to 3,750 m.

We consider that the long range locations of large mammals are accompanied by degrees of error which can not be disregarded, however, when radiotelemetry is used for animal studies, there is rarely an opportunity to know the distance between an animal's real, and its estimated, location. Salts (1994) recommended that those using radiotelemetry should assess their degree of error with an appropriate method and should describe their area of error.

Andrews estimator was regarded as robust, particularly where reflected signals occurred frequently (Garrott *et al*. 1986). It was concluded, however, that Andrews estimator suffers the same extent of error as that estimated by the TCM, or even a significantly larger error than the TCM. Andrews estimator maintained accuracy by failing to generate location estimates when bearings did not adequately converge (Garrott *et al*. 1986). In our field data, it may be impossible to eliminate bad locations sufficiently, as many bearings were biased.

Zimmerman (1990) and Zimmerman and Powell (1995) showed that both the error polygon method (Heezen and Tester 1967) and Lenth's (1981) maximum likelihood estimator which is the origin of Andrews estimator were poor indicators for estimating location errors and they recommended an approach using the location error method (LEM). Error areas using this approach were indicated by circles with 90% and 95% confidence distances between estimated locations and true locations as their radii (Zimmerman and Powell 1995). Our approach was essentially the same as the LEM, and our results confirmed the superiority of this approach. We were able to realize the extent of our TCM location errors by field testing.

We urge that when researchers begin a radiotelemetry study, they measure their location precision in the field. They must then judge whether the degree of error that they record is acceptable or not. They should also describe their average location distances and the extent of errors in their reports.

In this study, we did not consider possible increases of error resulting from animal movements. Shumutz and White (1990) calculated such errors by computer simulation. We should take these errors into account by adding error or by decreasing the time interval of measurement.

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Food habits of sympatric insectivorous bats in southern Kyushu, Japan

Kimitake Funakoshi[1] and Yuki Takeda[2]

[1]*Biological Laboratory, Kagoshima Keizai University, Kagoshima 891-0191, Japan*
Fax. +81-99-261-3299, e-mail. funakoshi@soc.kkis.ac.jp
[2]*Department of Biology, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan*
(Present address : *Yakusugi Museum, Yaku-cho 891-4311, Japan*)

Abstract. Five species of bats, *Myotis nattereri, M. macrodactylus, Miniopterus fuliginosus, Rhinolophus ferrumequinum* and *R. cornutus* were found to forage in the same habitats in southern Kyushu, Japan. *M. nattereri* fed mainly on Lepidoptera, Coleoptera, Diptera and Araneae, the proportions of each of these in the diet fluctuating seasonally, however, Lepidoptera and Coleoptera, especially, were consumed selectively. Their available prey items ranged in body length from 5–13 mm in length. *M. macrodactylus* preyed mainly on Diptera, Trichoptera and Lepidoptera, that were larger (7–20 mm) than those eaten by *M. nattereri*. Small or medium-sized Lepidoptera constituted the bulk of *M. fuliginosus'* diet in summer. *R. ferrumequinum* fed chiefly on larger Diptera, Coleoptera and Lepidoptera measuring 8–45 mm in body length, and clearly selected beetles despite these being relatively few in the trap samples. Lepidoptera and Diptera measuring 7–23 mm were important dietary components for *R. cornutus*, and despite their abundance being relatively low in summer moths were selectively preyed upon. These five bat species selectively hunted particular prey species in addition to taking food opportunistically. Through differences in both foraging-site and in prey selection, they seem to be able to coexist in the same habitat.

Key words : fecal analysis, food habits, insectivorous bats, prey selection, resource partitioning.

Food and roosts are potentially limiting resources that may affect the community structure of bats (Findley 1993), consequently, studies of foraging ecology and behavior may provide an insight into mechanisms that have permitted local coexistence in bat communities by reducing or eliminating competition (Kunz 1973). Information about prey selection and resource partitioning is very important for an understanding of how sympatric species of bats can coexist. Insectivorous bats are particularly interesting subjects for such studies because both the availability of insects to them is readily monitored and their actual diets can be determined by fecal analysis (Whitaker 1988).

Despite this, little is known about the resource partitioning or dietary overlap of sympatric insectivorous bat species (Black 1974, Swift and Racey

1983, Hickey *et al*. 1996), and in fact relatively few studies have examined the relationship between the prey actually eaten by bats and the abundance of available insects (Black 1974, Funakoshi and Uchida 1975, 1978, Anthony and Kunz, 1977, Swift *et al*. 1985, Lacki *et al*. 1995).   In Japan, there have been very few detailed studies of the food habits of insectivorous bats (Kuramoto 1972, Funakoshi and Uchida 1975, 1978).

Nothing was previously known of the food habits of either *Myotis nattereri* or *M. macrodactylus* in the field, thus here we report the first information on the dietary composition of these two species, and in addition we evaluate prey selection by *M. nattereri, M. macrodactylus, Miniopterus fuliginosus, Rhinolophus ferrumequinum* and *R. cornutus* in relation to food availability, and we examine whether resource partitioning occurs among these sympatric species.

Materials and Methods

Principal investigations were made in and around the tuffaceous Katano-dō Cave in Kagoshima Prefecture from the spring of 1994 to the fall of 1995. The vegetation of this region consists of secondary laurel forests and coppice forests, with fields on the western side and *Cryptomeria japonica* plantations to the south.   A small brook flows near the entrance of the cave.   *M. nattereri, M. macrodactylus, M. fuliginosus, R. ferrumequinum* and *R. cornutus* were all found at the cave from spring to fall (Funakoshi 1988, 1991).   Bats were captured using either insect sweep nets or mist nets.   Their sex and age were noted, and each bat was marked with a wing-band then released.   We collected 30–50 fresh feces under the roosting sites of the colonies of each species in the cave and by placing captured bats into holding bags.   Feces of *M. nattereri* and *R. ferrumequinum* in particular were collected every month to detect seasonal changes in their diets.

Insects were collected during the study period, using an insect suction trap (Tokyo AS Co. Ltd. Model DC-12) and a light trap with 20-watt white and black lights at 30 min intervals from dusk until dawn in the area where bats were foraging.   Feces were collected occasionally from Obirano-dō and Nakadake-dō Caves which are situated near Katano-dō Cave (Funakoshi 1988).   The vegetation around all three caves is similar.   *M. nattereri, M. fuliginosus* and *R. ferrumequinum* were found in Obirano-dō and Nakadake-dō Caves from spring to fall, and *M. macrodactylus* and *R. cornutus* were found there occasionally.   Data from fecal samples at Katano-dō Cave were supplemented with samples from Obirano-dō and Nakadake-dō Caves.

Insects from the suction trap samples, were identified and the proportion of each insect order was determined.   Several insects from each family caught at the light traps were crushed with forceps and keys were compiled from the fragments in order to assist in the identification of insect parts recovered from bat feces.   All feces were examined under a binocular microscope.   Recognizable fragments were extracted, and were identified with reference to the keys. The frequency of occurrence of each order of insects in fecal components was

given as a ratio of the number of the feces including one or more fragments of a certain order of insects to the sum of the number of feces in which one or more fragments were found for each order.

RESULTS

1. Seasonal changes in population size

In spring, *M. nattereri*, *M. macrodactylus*, *M. fuliginosus* and *R. ferrum-equinum* moved into Katano-dō Cave from their hibernaculae (Fig. 1). Before parturition in May and June, the *M. nattereri* and *M. macrodactylus* colonies consisted almost entirely of pregnant females (80 *M. nattereri*, and 50 *M. macrodactylus*). By the weaning season, the number of *M. nattereri* had increased to 150 and *M. macrodactylus* to 80. During late November, they emigrated. In summer, the *M. fuliginosus* colony consisted of 2,000–4,000 adult and subadult males, and subadult females (Fig. 1). During October that number diminished to about 1,000, and thereafter the remainder emigrated. In June, the colony of *R. ferrumequinum* consisted almost entirely of adult females, and their number was 150 (Fig. 1). During July, the lactating season, the colony attained its maximum size of 300 adult and subadult females, and young bats. A total of 120 *R. cornutus* hibernated at Katano-dō Cave (Fig. 1). Their number diminished during April, increased by 40–80 in May-July, then decreased again during August-October. Most of them were adult and subadult
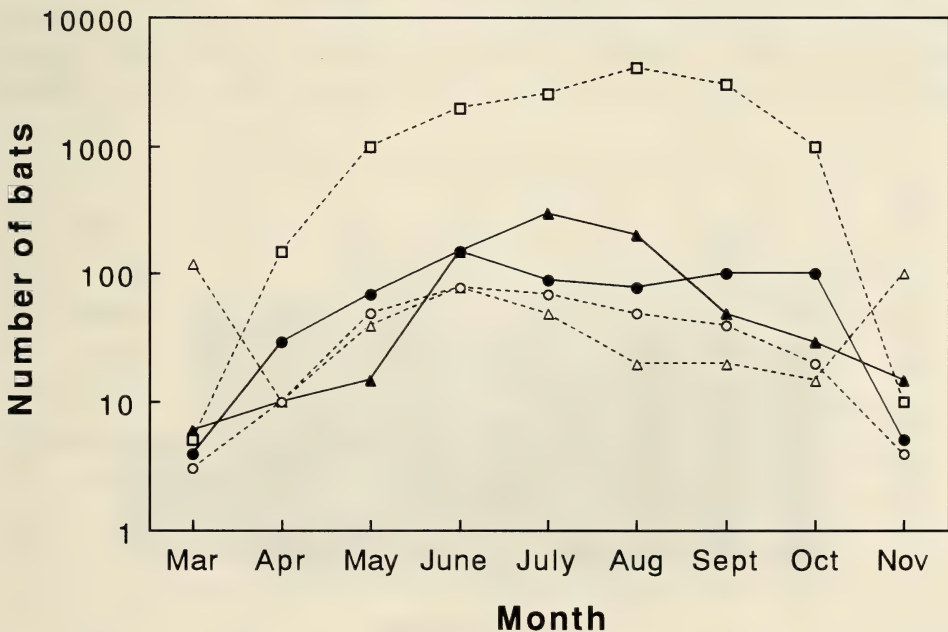
Fig. 1. Seasonal changes in the numbers of *R. cornutus* (---△---), *R. ferrumequinum* (—▲—), *M. macrodactylus* (---○---), *M. nattereri* (—●—) and *M. fuliginosus* (---□---) at Katano-dō Cave in 1994.

males and subadult females.

2. Food habits

Five orders of volant insects : Diptera, Lepidoptera, Coleoptera, Trichoptera and Ephemeroptera, as well as spiders (Araneae) were represented in the diet of *M. nattereri* (Fig. 2).   The body lengths of the available prey were 5-13 mm (Table 1).   Among the taxa commonly preyed on by *M. nattereri* were *Sericania* (Scarabaeidae, body length *ca.* 11 mm), *Macrolagria rufobrunnea* (Lagriidae, *ca.* 10 mm), Carabidae (*ca.* 10 mm), Tipulidae (8-13 mm), Araneidae (6-8 mm), *Tetragnatha* (Tetragnathidae, 8-10 mm) and Theridiidae (*ca.* 6 mm). The frequency of the occurrence of Diptera in *M. nattereri* feces fluctuated between 15 and 39% from April to November (see Fig. 2).   The frequency of Lepidoptera was 15-26% from April to August, but increased to 33-50% in fall. The frequency of Coleoptera was 42% in April, but dropped to 8% in May before increasing to 18-27% in summer, and then falling to 7-21% in fall.   The occurrence of Trichoptera and Ephemeroptera was less than 13% from April to November, while the Araneae varied from 3-48% from April to November, peaking 40-48% in May-June.

The prey of *R. ferrumequinum* included Diptera, Lepidoptera, Coleoptera, Trichoptera and Plecoptera (Fig. 3), ranging in size from 8 to 45 mm (Table 1). Species or genera that were frequently found in the diet included : *Tipula coquilletti*, other Tipulidae and *Tabanus* (Diptera, 14-30 mm in body length) ; *Holotrichia picea*, *Anomala cuprea*, *A. geniculata*, *A. daimiana*, *A. albopilosa*, *Melolontha japonica*, *M. satsumaensis*, *Mimela splendens*, *M. costata*, *Maladera castanea*, *M. secreta*, *Hydaticus grammicus*, *Melanotus legatus* and *Prionus insularis* (Coleoptera, 10-45 mm long).   The frequency occurrence of Diptera
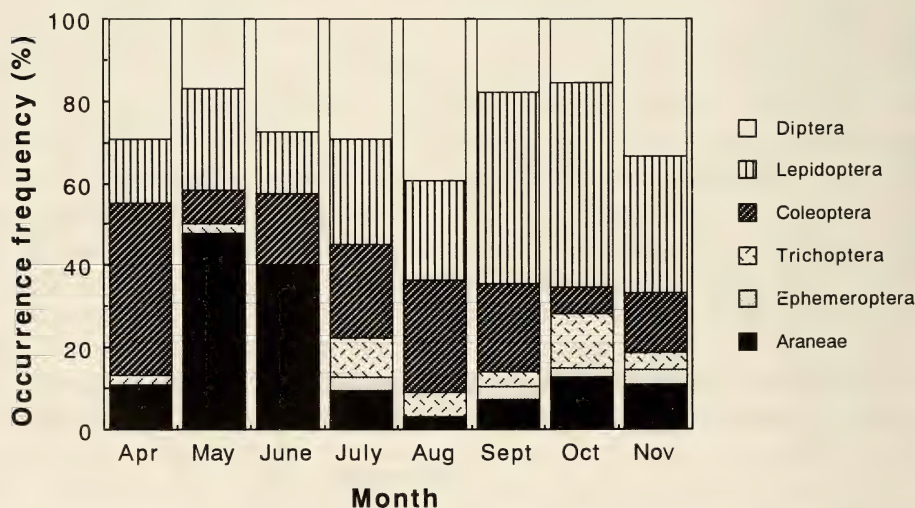
Fig. 2.   Seasonal changes in occurrence frequency of foods (order level of insects) in the feces of *M. nattereri* in 1994-1995.

Table 1. Body length for each order of insects found in fecal pellets of bats.

Bat species	Prey item	Body length (mm)
Myotis nattereri	Coleoptera	6−12
	Lepidoptera	8−13
	Diptera	5−11
	Trichoptera	8−10
	Ephemeroptera	7−12
Myotis macrodactylus	Coleoptera	8−18
	Lepidoptera	7−15
	Diptera	8−20
	Trichoptera	8−11
	Ephemeroptera	9−12
Miniopterus fuliginosus	Coleoptera	6−15
	Lepidoptera	6−25
	Diptera	7−22
	Trichoptera	5−10
	Ephemeroptera	10−12
Rhinolophus cornutus	Coleoptera	8−22
	Lepidoptera	9−23
	Diptera	7−23
	Trichoptera	8−10
Rhinolophus ferrumequinum	Coleoptera	9−45
	Lepidoptera	12−27
	Diptera	10−30
	Trichoptera	8−12
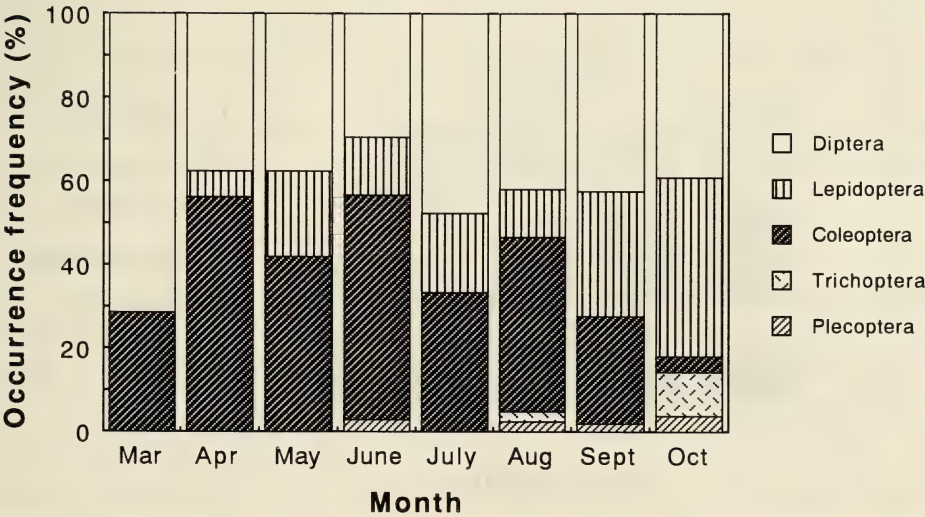
Fig. 3. Seasonal changes in occurrence frequency of foods (order level of insects) in the feces of *R. ferrumequinum* in 1994–1995.

in *R. ferrumequinum* feces was 71% during March, but fluctuated between 30 and 48% from April to October (Fig. 3).   The frequency of Coleoptera in the diet varied between 26 and 56% from March to September, and dropped to 4% in October, whereas the frequency of Lepidoptera gradually increased from April onwards reaching 43% in October.   The frequency occurrence of both Trichoptera and Plecoptera was less than 11% from spring to fall.   Most significant was that the combined frequency occurrence of both Diptera and Coleoptera was 80% or more from May to August.

The diet of *M. macrodactylus* included Diptera, Lepidoptera, Coleoptera, Trichoptera, Plecoptera, Ephemeroptera and Araneae, ranging in size from 7 to 20 mm in body length (Table 1).   These bats commonly took for example : Tipulidae (8-20 mm in body length), Tabanidae (*ca.* 18 mm), Scarabaeidae (*A. geniculata ca.* 12 mm, *A. daimiana ca.* 16 mm, and *H. picea ca.* 18 mm), and Araneidae (*ca.* 10 mm).   The frequency occurrence of various prey from March to May as determined by analysis of *M. macrodactylus* feces were : Diptera 45 %, Trichoptera 18 %, Coleoptera 9 %, Plecoptera 9 %, Ephemeroptera 9 %, Lepidoptera 5 % and Araneae 5 %.   In July these frequencies changed to : Diptera 36 %, Trichoptera 16 %, Coleoptera 16 %, Lepidoptera 24 % and Araneae 8 % (Fig. 4).

The prey of *M. fuliginosus* in July, as measured by fecal analysis, included Diptera 23 %, Lepidoptera 44 %, Coleoptera 7 %, Trichoptera 14 %, Ephemeroptera 7 % and Plecoptera 5 % (Fig. 4), ranging in size from 5 to 25 mm in body length (Table 1).   For example, the Tipulidae (Diptera) that were eaten measured, *ca.* 10 mm and *A. geniculata* (Coleoptera) *ca.* 12 mm.
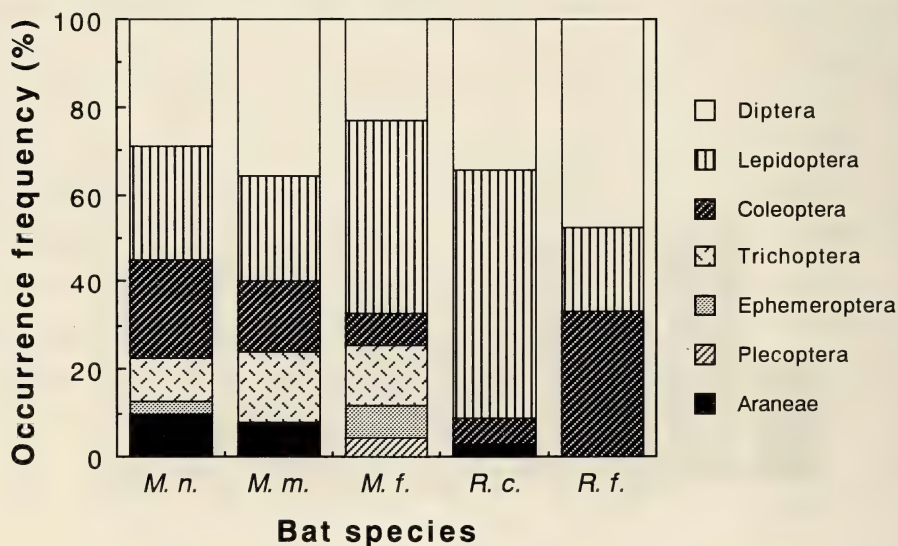
Fig. 4.   Occurrence frequency of foods (order level of insects) in the feces of *M. nattereri* (*M. n.*), *M. macrodactylus* (*M. m.*), *M. fuliginosus* (*M. f.*), *R. cornutus* (*R. c.*) and *R. ferrumequinum* (*R. f.*) in July of 1994.
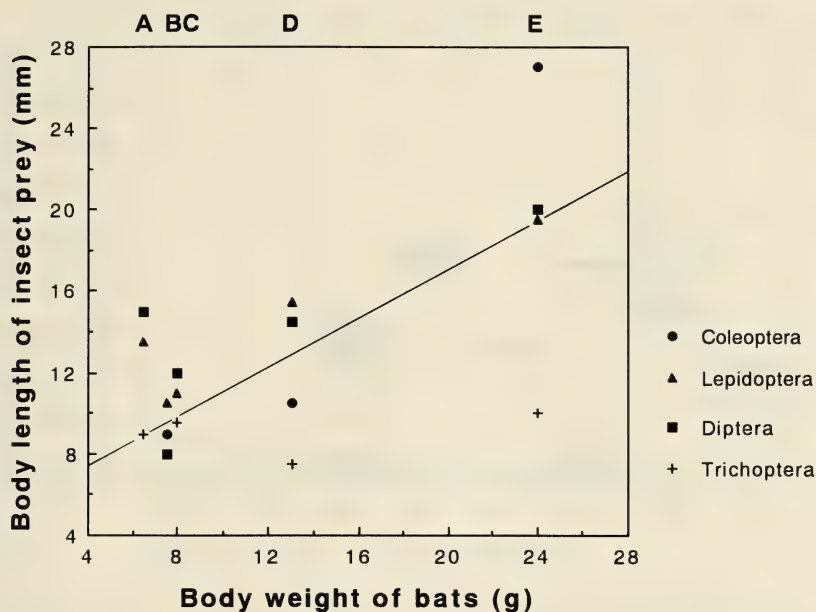
Fig. 5.   Correlation of mean body weight of bats and mean body length of insect prey.   A :
R. cornutus, B : *M. nattereri*, C : *M. macrodactylus*, D : *M. fuliginosus*, E : *R. ferrumequinum*.

The diet of *R. cornutus* in July included Diptera 34 %, Lepidoptera 57 %,
Coleoptera 6 % and Araneae 3 % (Fig. 4), ranging in size from 7 to 23 mm in
body length (Table 1).   In addition to these orders, Trichoptera was found in
their feces in June.   Typical examples were Tipulidae (Diptera) measuring 8-
23 mm, and *M. castanea, A. geniculata* and *H. grammicus* (Coleoptera), measur-
ing 9-15 mm.

A significant positive relationship was found between the mean body
weight of the bats and the body length of their insect prey (Pearson's correla-
tion coefficient : $r = 0.63$, $p < 0.01$, see Fig. 5).

3. Insect abundance

Total insect numbers (collected at the night) reached a peak during July,
whereas dry weights were heaviest during June (see Figs. 6 and 7).   In every
month Diptera constituted a major portion of these samples (Fig. 6), but the
ratio of the dry weight of Diptera to that of all insects trapped from April to
September was less than 25 % (Fig. 7).   Trichoptera (4-25 %) and Ephemero-
ptera (1-21 %) were the next most abundant groups from April to November
(Fig. 6), although the ratios of their dry weight to those of all insects was less
than 16 % for the Trichoptera, and less than 10 % for the Ephemeroptera (Fig.
7).   Lepidoptera and Coleoptera were often collected, but constituted only a
small percentage of the total fauna (Fig. 6), yet the two orders contributed a
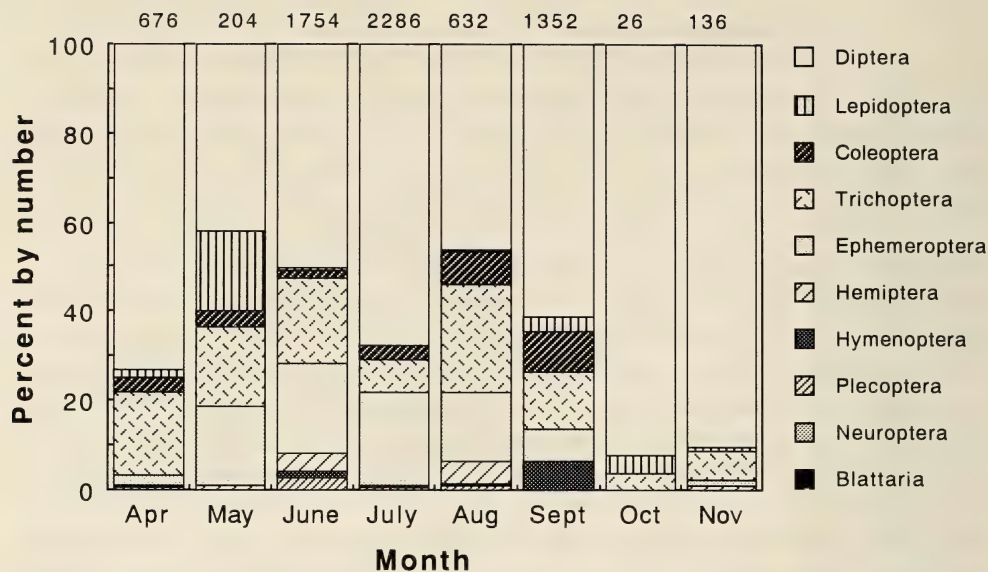
Fig. 6. Seasonal changes in percent number of insects of various orders collected by insect suction traps near Katano-dō Cave in 1994. Monthly sample sizes are indicated above histograms.
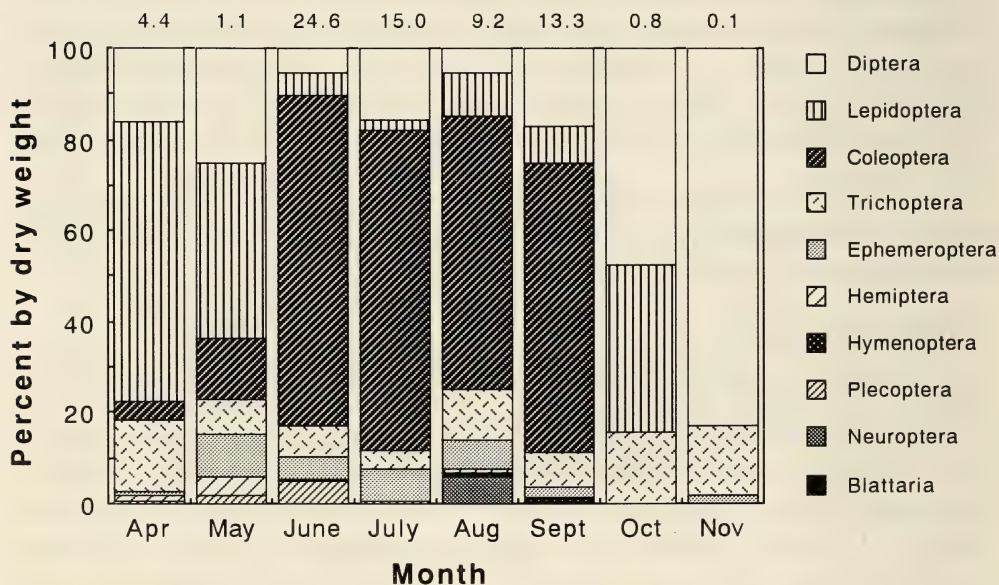
Fig. 7. Seasonal changes in percent dry weight of insects of various orders collected by insect suction traps near Katano-dō Cave in 1994. Monthly total dry weights (g) are indicated above histograms.
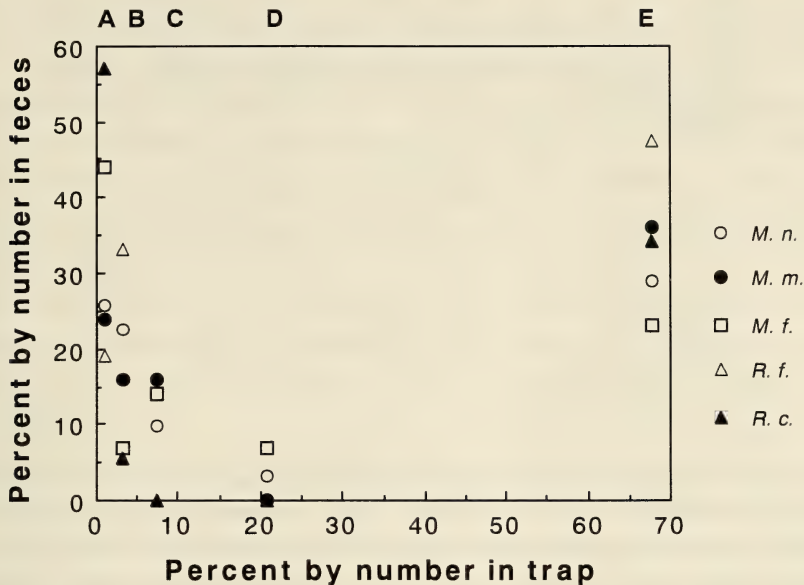
Fig. 8.   Percent representation of each of five groups of insects in the feces of bats and in insect suction traps.   A: Lepidoptera, B: Coleoptera, C: Trichoptera, D: Ephemeroptera, E: Diptera; *M. n.*: *M. nattereri*, *M. m.*: *M. macrodactylus*, *M. f.*: *M. fuliginosus*, *R. f.*: *R. ferrumequinum*, *R. c.*: *R. cornutus*.

high proportion of the dry weight.   The ratio of the dry weight of Lepidoptera to that of all insects varied from 2 to 61 % from April to October, being particularly high (37-61 %) during April, May and October (Fig. 7).   The ratio of dry weight of Coleoptera to that of all insects fluctuated even more widely between 4 and 73 % from April to September, being particularly high (60-73 %) from June to September (Fig. 7).

When the rates of occurrence in bat feces of the five main insect groups were compared with the same groups occurring in traps during July, no correlation was found between the two values (Pearson's correlation coefficient: $r = 0.30$, $p > 0.1$, see Fig. 8).   The percentages of Diptera in the feces of both *M. nattereri* and *R. ferrumequinum* were significantly lower than those in the trap samples (Tables 2 and 3), whereas the percentages of Lepidoptera or Coleoptera in the feces were significantly larger.

In addition, spiders including Araneidae, Tetragnathidae and Theridiidae were frequently found along the edges of woodlands or brooks during May and June, however quantitative samples were not collected.

DISCUSSION

1. Prey selection

M. nattereri was found to feed mainly on Lepidoptera, Coleoptera, Diptera

Table 2. Proportions of five insect groups in insect suction traps and in the feces of *M. nattereri* for eight nights. Probability (*p*) refers to Wilcoxon signed-ranks tests applied to establish whether each insect group was consistently commoner or rarer in the trap samples than in the fecal ones.

Order	% trapped Mean±SD	% in feces Mean±SD	*p*
Diptera	65.4±19.2	32.1± 9.8	<0.01
Lepidoptera	3.5± 5.9	36.2±14.4	<0.01
Coleoptera	3.4± 3.3	24.1±11.9	<0.01
Trichoptera	13.9± 7.4	5.8± 4.8	N. S.
Ephemeroptera	10.7± 8.9	1.8± 1.9	N. S.

N. S. : not significant.

and Araneae, whereas aquatic insects such as Trichoptera and Ephemeroptera contributed smaller proportions of their diet.   Lepidoptera and Coleoptera, in particular, were consistently selected by *M. nattereri* (Fig. 2 ; Table 2).   Their predatory habits were probably a direct consequence of their particular preference for foraging in woodlands rather than over or near water, and this in turn was presumably related to their wing structure, as woodland foraging *M. nattereri* has relatively broader wings than does its close relative *M. macrodactylus* (Kuramoto 1972, Funakoshi 1988).   Similarly, *Myotis auriculus* and *Plecotus auritus*, both of which have long ears, also prey mainly upon moths and beetles (Husar 1976, Swift and Racey 1983).   *P. auritus* has relatively broader wings and a lower aspect ratio making it more maneuverable than *M. auriculus*, which characteristics make it possible for it to fly and hover skillfully (Norberg 1970), and hence to forage in thickly wooded areas (Swift and Racey 1983).

Spiders (Araneae) are a particularly important component of *M. nattereri's* diet, especially during May and June (Fig. 2), and they have also been found to be eaten by *Nycteris thebaica*, *P. auritus*, *Plecotus townsendii virginianus* and

Table 3. Proportions of five insect groups in insect suction traps and in the feces of *R. ferrumequinum* for seven nights. Probability (*p*) refers to Wilcoxon signed-ranks tests applied to establish whether each insect group was consistently commoner or rarer in the trap samples than in the fecal ones.

Order	% trapped Mean±SD	% in feces Mean±SD	*p*
Diptera	61.8±17.6	39.4± 5.6	<0.01
Lepidoptera	3.9± 6.2	20.6±12.4	<0.01
Coleoptera	3.9± 3.2	36.6±18.1	<0.01
Trichoptera	15.0± 7.3	1.9± 4.0	N. S.
Plecoptera	0.5± 0.9	1.5± 1.5	N. S.

N. S. : not significant.

Myotis grisescens, although they contribute only very small percentages to their diets (LaVal and LaVal 1980, Swift and Racey 1983, Sample and Whitmore 1993, Best *et al.* 1997). Swift and Racey (1983) have even suggested that *P. auritus* may glean for spiders, however as some spiders were attached to gossamer they would also have been available to bats in full flight. In contrast, *M. nattereri, M. macrodactylus* and *R. cornutus* may catch spiders chiefly in flight, because nearly all of the spiders eaten were found to be snarers. It does seem that spiders may be consumed opportunistically by all these bats, when they were abundantly available.

In our study area *M. nattereri* foraged mainly in woodlands and ate smaller Lepidoptera, Coleoptera, Diptera and Araneae than other bats did (Table 1), whereas *M. macrodactylus* foraged not only in woodlands but also over or near water (Kuramoto 1972) and fed mainly on medium-sized Diptera, Trichoptera or Lepidoptera that were larger than those eaten by *M. nattereri* (Table 1). As a consequence of their preferred riparian foraging habitat, *M. macrodactylus* often fed on aquatic insects such as Trichoptera, Ephemeroptera and Plecoptera. Another species foraging very similarly is *Myotis daubentoni*, which flies almost entirely above water and riparian vegetation and feeds mainly on Diptera and Trichoptera (Swift and Racey 1983).

M. fuliginosus has relatively long-narrow wings and a high aspect ratio, enabling it to fly quickly (Kuramoto 1972). It prefers to forage above the woodland canopy or over water, where in summer it prefers small or medium-sized moths (Kuramoto 1972, Funakoshi and Uchida 1975 ; see Table 1, Figs 4 and 8). Other populations of this species in Kumamoto Prefecture, Kyushu, have also been shown to consume largely Lepidoptera (Funakoshi and Uchida 1975). During spring or fall, however, they also feed opportunistically on insects, particularly on Diptera Trichoptera and Ephemeroptera (Funakoshi and Uchida 1975).

R. ferrumequinum is a relatively large bat with short, broad wings, capable of making short low speed flights and even hovering, which prefers to forage not only near or in thick woodlands but also in open spaces over water or over grasslands. They may also pursue prey on the ground (Kuramoto 1972). They feed mainly on relatively large insects such as Diptera, Coleoptera and Lepidoptera (Kuramoto 1972, this paper), the proportions in the diet changing seasonally (see Fig. 3, and Jones 1990). Beetles in particular were selectively consumed from April to September in Japan, even though their numbers in the trap samples were small (Table 3, Fig. 8), whereas moths constitute a major portion of this species' diet throughout the summer in England (Jones 1990). These bats, thus, probably select prey by size rather than by order during periods of abundance, and they also may engage in opportunistic feeding at times depending on prey availability and abundance. Opportunistic feeding by bats allows effective exploitation of patchily distributed food resources and can lead to selective feeding (Fenton and Morris 1976).

R. cornutus also has short-broad wings, giving it a very low wing-loading and low aspect ratio which enable it to fly or hover slowly and to roll rapidly

while foraging near or in thick woodland and in open spaces (Kuramoto 1972). Lepidoptera and Diptera were found to be the first and second most important dietary items for *R. cornutus*, however they took smaller prey than *R. ferrum-equinum* did (Fig. 4, Table 1). Despite their relatively low abundance during July, *R. cornutus* fed selectively on moths (Figs 6, 7 and 8).

2. Resource partitioning

Spatial partitioning, with *M. fuliginosus* hunting in open spaces far above the woodland canopy or over water, ensures that there is little or no competition for food resources with the other four sympatric species which forage within or around foliage and in the open spaces between trees. Intraspecific competition for food may exist, however, in *M. fuliginosus* because they occur at high densities (Fig. 1).

As previously mentioned, *M. nattereri* and *M. macrodactylus* forage in partially different habitats, and feed on different sized insects. Their different prey sizes probably reflect the differences in their body sizes, and these differences may weaken niche overlap or competition between these two closely related and sympatric species. Two other similar-sized bats, *P. auritus* and *M. daubentoni*, are also known to partition resources in space and to eat different types of prey (Swift and Racey 1983), and *M. auriculus* and *M. evotis*, which closely resemble each other, and which have similar food habits when occurring allopatrically, avoid competition for food when occurring sympatrically by *M. evotis* changing its food preferences (Husar 1976).

R. ferrumequinum and *R. cornutus* are morphologically similar, but the former is about three times larger than the latter. The two species were found to forage in similar places, but consumed insects from different orders and of different sizes, thus is little overlap in their diets (Kuramoto 1972, this study). Similarly, *Lasiurus cinereus* is about twice as large as *L. borealis*, and where their ranges overlap, the former primarily eats larger moths while the latter eats smaller moths (Acharya and Fenton 1992, Hickey *et al.* 1996). The study colony of *R. cornutus* was relatively small (Fig. 1), and its members fed mainly on Lepidoptera which were less common in the trap samples in our study area (Fig. 6). The low abundance of preferred prey may be correlated with the small population size in summer.

The foraging areas of *R. ferrumequinum*, *M. nattereri* and *M. macrodactylus* partially overlap, thus competition for foods is perhaps being avoided by the differences in the size of their prefered prey. In particular, the larger *R. ferrumequinum* tended to eat larger insects such as chafers and gadflies. Similarly, niche overlap partially occurs between *R. cornutus* and *M. nattereri* or *M. macrodactylus*, however *R. cornutus* tends to emerge after sunset about 30 min earlier than the other species (Kuramoto 1972, also this study). As the main emergence time of insect prey was found to be within a few hours after sunset (Funakoshi and Takeda unpublished), foraging during this period may be very important for them to consume insects efficiently. It is probable that resource partitioning between them may occur in the same foraging areas

through differences in both their prey selection and in their temporal activity.

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Review

The present status, ecology and conservation of the Mongolian gazelle, *Procapra gutturosa*: a review

Jiang Zhaowen[1], Seiki Takatsuki[2], Gao Zhongxin[3] and Jin Kun[3]

[1]*Laboratory of Wildlife Biology, School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan*
Fax. +81-3-3815-7053, e-mail. jiang@um.u-tokyo. ac. jp
[2]*The University Museum, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan*
[3]*College of Wildlife, Northeast Forestry University, 26 Hexing Road, Harbin 150040, P. R. China*

Abstract. The grassland ecosystem of Mongolia and adjacent areas of Russia and northeastern China is an important component of the natural ecosystems of Eastern Asia. This grassland ecosystem is unique in that it has been utilized for grazing for a long period of time without deterioration. The Mongolian gazelle, *Procapra gutturosa*, an important species occurring in this ecosystem, used to be abundant and widely distributed, however, populations have decreased in recent decades and the distribution of the species has become greatly reduced. The contraction of its range began in the early years of the 20th century in China, during the 1970s in Russia, where they completely disappeared, and after the 1950s in Mongolia where the majority of the population now remains. The total population has decreased from about 1.5 million heads in the 1940s to 300,000-500,000 at present. In Mongolia, their range spanned about 780,000 km² in the 1950s, but this has contracted to only 170,000 km² at present. Mongolian gazelles inhabit grasslands and eat mainly grasses such as *Stipa* spp. and *Aneurolepidium chinense*. During summer they occur in small groups of 20-30 individuals, and in winter usually of 100-120 individuals, although they sometimes gather into herds of several thousands during periods of snow. They migrate seasonally, but their routes and the distances travelled are unclear. Their reproductive capacity is high with very high pregnancy rates ranging from 80% to 100% among females older than 1.5 years. The present problems facing the population and the future needs for conservation are discussed with the main conclusion being that international cooperation for the establishment of reserves is urgently required.

Key words: China, Mongolia, Mongolian gazelle, *Procapra gutturosa*, Russia, wildlife conservation.

The wildlife living in the natural ecosystems occurring in developing countries faces severe difficulties, and many species are already either endangered or even close to extinction. There are many such examples in China.

It is well known that China's natural forests have been reduced rapidly during the latter half of this century. Natural forests, however, are not the only ecosystem in decline. Natural grasslands are also under serious threat. About 42% of China's land area is classified as grassland, more than half of which is in northern China (National Research Council 1992). In 1989, for example, grasslands still covered 70% of Inner Mongolia and supported more than 37 million livestock. Eastern Inner Mongolia, adjacent to Russia and Mongolia, consists predominantly of that part of the Mongolian plateau known as the Hulunbeier plateau grasslands. This grassland ecosystem has been degraded because of human expansion, agricultural development and overgrazing since the 1960s. As a consequence, wildlife populations and their ranges have been seriously reduced. In particular, ungulates and their predators, such as wolves, have been greatly reduced by human activity.

Though there have been relatively many studies of the vegetation and plant ecology of these grassland ecosystems by Chinese scientists, animal ecology has received less attention except for the ecology of rodents because of their impact on grassland productivity. As a consequence, little is known of the wildlife of the grasslands. Understanding the interrelationships between wild ungulates and livestock is important in order to promote better management of these grasslands.

Among the wild ungulates of this region, the Mongolian gazelle, *Procapra gutturosa*, used to be the most numerous and was a significant component of the grassland ecosystem. The population has, however, decreased dramatically and now faces extinction in China.

It is very important, therefore, to develop a conservation strategy, and this should take account not only of the conservation of the Mongolian gazelle itself but also the management of the grassland ecosystem so as to facilitate the coexistence of wild gazelles and domestic livestock.

Although there has not previously been a review of the information available on the Mongolian gazelle, a number of papers on the species has been published. One reason for *P. gutturosa* being so poorly known in the Western World is that most literature on the species has been published either in Chinese or in Russian. We have tried, therefore, in this paper to rectify that situation by referring to as much of this literature as possible. Because of the limited availability of some of the Russian literature, however, some literature has been cited from abstracts. It is hoped that this review will help to provide the necessary information required to construct a conservation strategy for the future of this species.

GENERAL DESCRIPTION AND TAXONOMY

The genera *Gazella*, *Saiga* and *Naemorhedus* are all closely related, and formerly the *Procapra* were even included within the genus *Gazella*, however, most taxonomists now agree on placing the *Procapra* in their own genus. Ellerman and Morrison-Scott (1951, 1966) only recognized two species in the

genus, but today three are recognized (Corbet 1978), these are: the Mongolian gazelle, the Tibetan gazelle, *P. picticaudata*, and Przewalski's gazelle, *P. przewalskii*.

Adult Mongolian gazelles measure from 1 to 1.3 meters from head to rump, and stand about 75 cm high at the shoulder. Males weigh about 30 kg and females 25 kg. The summer coat is orange-buff, the flanks are pinkish cinnamon, and the belly is white with a long-haired dewlap. The winter coat is paler. During the rut, the males have swollen throats. Only males have horns and they range in length from 255 to 355 mm (Walker 1975, Jiang *et al.* 1991).

Fawns are born in May or June, weigh 2.8–3.0 kg, and measure 51–56 cm from head to rump (Гептнер *et al.* 1961). New-born lambs begin grazing about 10 days after birth, and grow quickly so that they attain weights of about 19 kg by six months of age. By one month old their body lengths are 74–82 cm, and by late September they have doubled in size since birth. Horns begin to appear when males are about four months old and reach full size when they are one year old (Bannikov 1954). Males and females both reach virtually full adult body size at 1.5 years old (Jiang *et al.* 1991), at which age females reach sexual maturity. Males, however, according to Lhagvasuren and Milner-Gulland (1997), reach sexual maturity at about 2.5 years old, however Гептнер *et al.* (1961) reported them breeding at just 17–18 months of age.

Soma *et al.* (1979, 1980), who studied the Giemsa banding pattern of the chromosomes of the Mongolian gazelle, found that $2n=60$. Soma *et al.* (1980) concluded that these banding patterns closely resembled those of the goral, *Naemorhedus goral*. Analyses of the karyotypes of the saiga, *Saiga tatarica*, and the Mongolian gazelle have shown that they too are closely related (Soma *et al.* 1979).

Allen (1940) thought that two subspecies, the Mongolian gazelle, *P. g. gutturosa* (Allen 1938) and the Altai gazelle (*P. g. altaica* Hollister 1913) could be distinguished. Allen's (1940) classification was based, however, on differences in the lengths of the horns and in the ratio of the distance between the horn tips, over the distance between the horn bases. This ratio changes, however, with age, thus it is difficult to distinguish the "subspecies" using it. Furthermore, the "subspecies" often live sympatrically, leading Zhao (1963) to consider Allen's (1940) classification invalid.

DISTRIBUTION

Mongolian gazelles once occurred widely across northern China, in most areas of Mongolia and in southern areas of Russia.

1. China

During the 19th century and even until the beginning of the 20th century, Mongolian gazelles were still widely distributed in northern China (Figs. 1 and 2). The southern limit of their distribution was in the northern part of Hebei Province around Beijing at 41°N, 112°E, and they seem not to have reached as
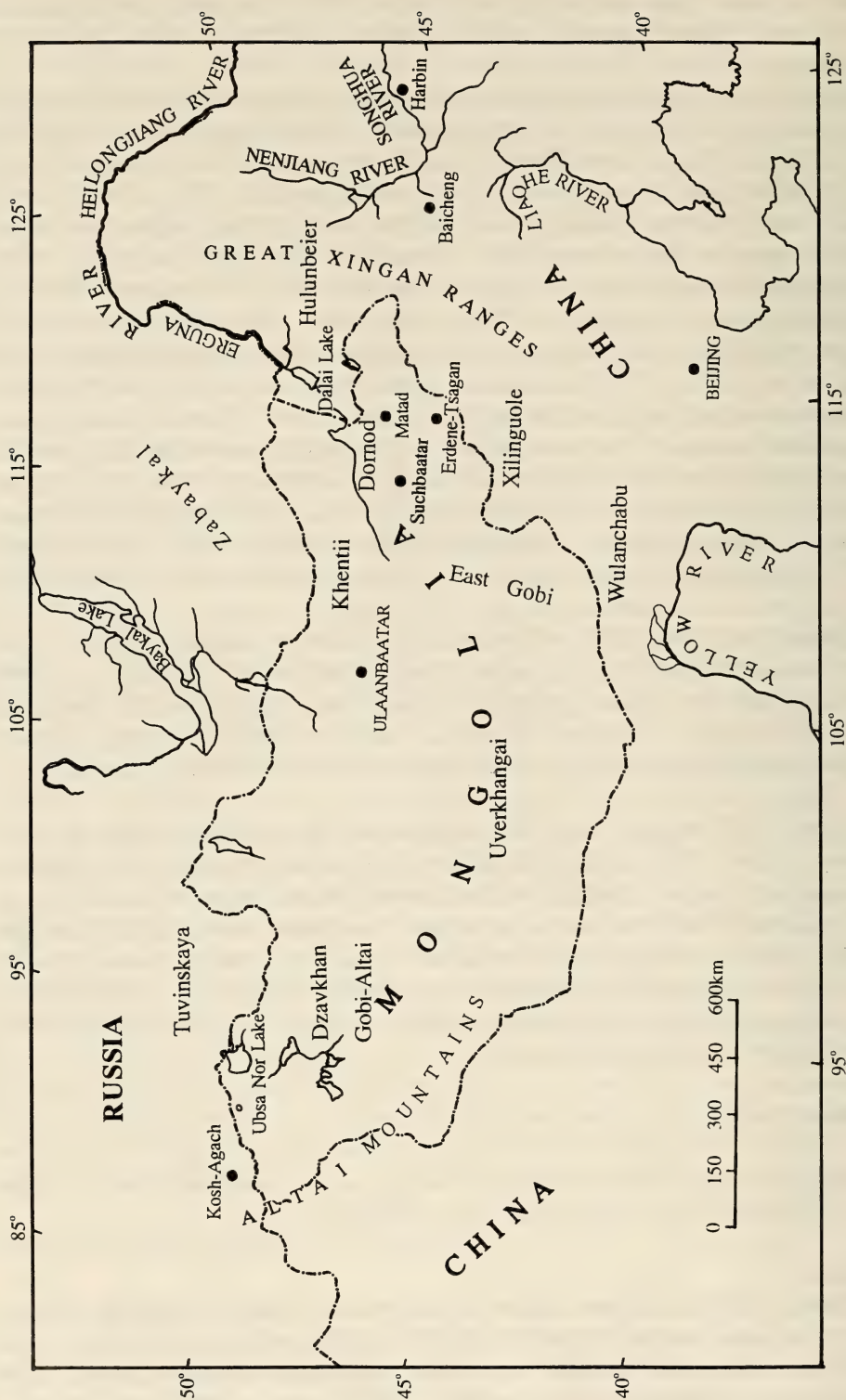
Fig. 1. Map showing the names of places relating to the distribution of the Mongolian gazelle.
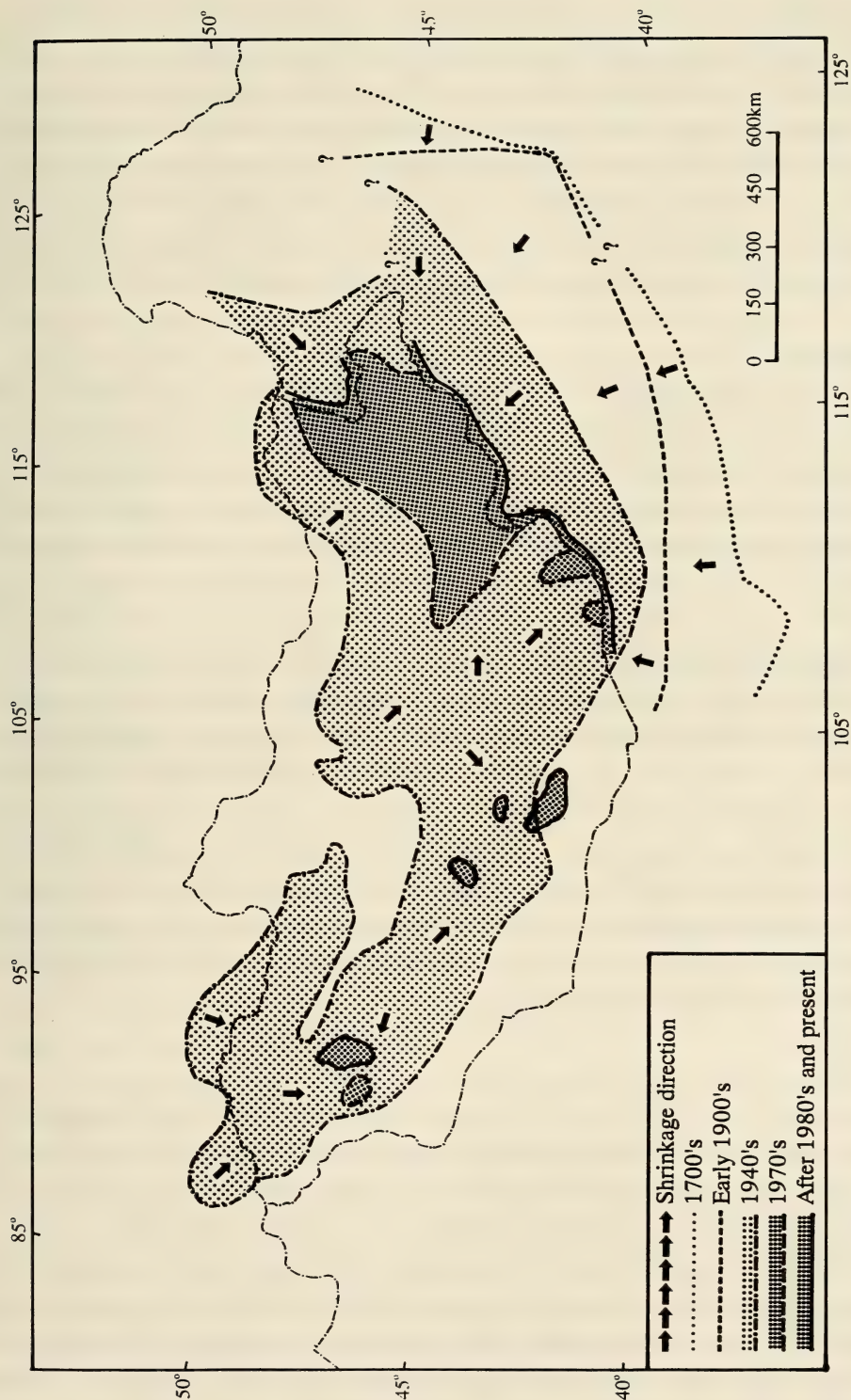
Fig. 2. The historical changes of the distribution of Mongolian gazelle in China and Mongolia.

far as the Yellow River (Лукашкин 1927). As agriculture has spread and developed, however, the gazelle's distribution has continuously shrunk. In the early decades of the 20th century, its range was still extensive (Figs. 1 and 2) : it occurred in Inner Mongolia across the Great Xingan Ranges, southward to the Nenjiang River and to the Songhuajiang River area of Heilongjiang Province, and to Baicheng in Jilin Province. The easternmost point of its range was reached in the middle of the watershed of the Liaohe River. At present, its southeastern limit is to be found in the leagues of Hulunbeier, Xilinguole and Wulanchabu at the border between China and Mongolia (Zhang *et al*. 1995). It is extinct now in Heilongjiang Province.

2. Mongolia

Most Mongolian gazelles are now to be found only in Mongolia. As recently as the 1950s, Mongolian gazelles were distributed widely across about two thirds amounting to about 780,000 km² of the country, though they were not to be found in northern forested areas or in southern desert areas, and the population still numbered about a million individuals (Bannikov 1954). During the 1950s, the northern limits of their range were reached in the Ubsa Nor lake basin (50°N, 91°E, Гептнер *et al*. 1961), however, during the second half of the 20th century, the range of the species in Mongolia has been shrinking. A survey conducted during the decade from 1975 to 1985 revealed that its distribution had decreased dramatically to just one quarter or one fifth (about 170,000 km²) of its range during the 1950s. Over the same period, the population decreased by half to about 500,000 animals. The remaining population was confined to areas such as : Khentii, Dornod, Suchbaatar and East Gobi in eastern Mongolia (Lushchekina *et al*. 1983, 1985, 1986, and Lhagvasuren and Milner-Gulland 1997). Small scattered populations remain in other parts of western Mongolia (Fig. 2, Lhagvasuren and Milner Gulland 1997). The most recent information (IUCN 1993) shows that most gazelles are now confined to an even smaller area (10% in size) in the eastern part of Mongolia and that the population amounts to only about 300,000 animals, of which about 60% are migratory and the rest sedentary.

3. Russia

Until the 1970s, Mongolian gazelles still occurred in small numbers in the southeastern Altai Mountains, southern Tuvinskaya and east Zabaykal of Russia (Figs. 1 and 2), though previously they had been common in some areas.

In the 19th century, several thousand Mongolian gazelles were to be found in east Zabaykal during winter (Черкасов 1867). During the winters of 1925-1926 and 1944-1945, several thousand Mongolian gazelles lived in east Zabaykal, while the number was fewer in west Zabaykal. During the winter of 1947-1948 only groups of fewer than 100 animals were found (Леонтьев 1949). In the grasslands of the southeastern Altai Mountains, hundreds of Mongolian gazelles were often found and large groups sometimes immigrated from Mongolia, but by the end of the 1950s the gazelles had become rare there

(Гептнер *et al.* 1961). In 1935 there were still several hundred gazelles in Tuvinskaya, but by the winter of 1940 only a few individuals remained, and thereafter no gazelles have been seen there (Янушевич и Благовещенский 1952). At Kosh-Agach and in the border area between Russia and Mongolia (Fig.1), only 5-6 gazelles were found in the 1956-57 winter (Гептнер *et al.* 1961). In west Zabaykal there were not many Mongolian gazelles and some of them migrated from Mongolia during winter. Their last visit was in the winter of 1947-1948, when they numbered fewer than 100 animals (Леонтьев 1949). In east Zabaykal the population was relatively stable until the 1940s, but during the 1950s the gazelles decreased and by the end of the 1950s only a few small groups were to be found in the border area of China, Mongolia and Russia (Гептнер *et al.* 1961).

Sludskii and Shubin (1963), who conducted aerial censuses of the Kazakh-stan Desert area in the winter of 1960, reported 9,300 gazelles, about 60% of which were in the northern part of the Kyzyl-Kum Sands (at about 60-70°E), though was, however, contrary to Гептнер *et al.* (1961) who defined the distribution of Mongolian gazelles in Russia as limited to areas east of about 85°E. Whatever the original limits of their distribution were, since the 1970s no gazelles have been seen in Russia, and it is believed that they are now extinct.

HABITAT

Information on the Mongolian gazelle's habitat in Mongolia and Russia is limited, and therefore information on the characteristics of their habitat is based on observations in China. Their main preference seems to be for flat or undulating steppes or dry grasslands.

1. Topography

The Great Xingan Range extends from northeast to southwest in the central Hulunbeier League (Fig. 1). To the west of the range is the rolling Hulunbeier Plateau which lies above 600 m. The highest point reached at 1,038 m is Bain Mountain, while the lowest place, the 2,200 km² Dalai Lake, is at 540 m. The areas around the Dalai and Beier Lakes, and along the Wuersun River are lowlands where rich water systems such as the Erguna River and Dalai Lake develop (Pan *et al* 1992).

2. Climate

Because the Great Xingan Range blocks the movement of moist oceanic winds, the climate here is semi-arid. The average annual temperature is as low as -3 to 0°C, while the lowest temperature reached is -40°C, and the highest 35-40°C. Continuous snow-cover lasts from 120 to 180 days each winter, and the frostless summer period is of 80-120 days. The annual rainfall is of only 250-380 mm, of which 70% falls in summer, while annual evaporation amounts to 1,300-1,900 mm (Pan *et al*. 1992). The main natural calamities that the gazelle's face in this region are snow, snowstorms and frostbite.

3. Vegetation

The vegetation which comprises typical gazelle habitat consists of cool temperate tall grassland (Hu *et al*. 1992).   Five types of such grasslands are recognized according to their species composition : 1) *Stipa grandis/Aneurolepidium chinense* type, 2) *Stipa grandis/Cleistogenes squarrosa* type, 3) *Cleistogenes squarrosa/Lespedeza* spp. type, 4) *Artemisia frigida* type and 5) *Aneurolepidium chinense/Stipa grandis/*Herbs type (Hu *et al*. 1992).

4. Other Animals

About 200 species of birds and more than 20 species of mammals have been recorded in the area (Office of Local Chronicles in Hulunbeier 1986).   Other mammals that are common in the area include bobak marmot, *Marmota bobak*, cape hare, *Lepus capensis*, steppe polecat, *Mustela eversmanni*, red fox, *Vulpes vulpes*, Corsac fox, *V. corsac*, the wolf, *Canis lupus*, and many species of mice.

FOOD HABITS

The Mongolian gazelle eats a wide range of plant species, however the bulk of its diet consists of a very limited number of species.   Bannikov (1954) identified just 21 plant species in the stomach contents of 22 gazelles from Mongolia.   These included : *Stipa capillata*, *S. gobica*, *Allium polyrrhyzum*, *Agropyrum pseudoagropyrum*, *Kochia prostrata* and *Koeleria gracilis*.   Interestingly, the gazelles avoid *Diplachne* spp. even though these are relatively abundant.   Of the 21 plant species recorded, *Stipa* spp. accounted for 60% of the stomach contents collected in January.   Bannikov (1954) found clear seasonal variation in diet with Gramineae, *Artemisia*, *Caragana*, *Allium* and Leguminosae in stomach contents sampled in spring, while in August about 80% of the stomach contents consisted of onions, *Allium* spp. (Lhagvasuren and Milner-Gulland 1997).

Fecal analyses of Mongolian gazelles in the Hulunbeier grasslands of Inner Mongolia, China during 1993-94 have revealed 38 plant genera in the diet with *Stipa* spp., *Aneurolepidium chinense*, *Caragana microphylla* and various Liliaceae and Compositae being of particular importance (Jin 1994, Gao *et al*. 1995).   In winter, the three main components of the diet were found to be *Stipa* spp. (38.6%), *A. chinense* (21.8%) and *C. microphylla* (7.5%).   In winter, the diet of the Mongolian gazelle is very similar to that of domestic sheep, the diet of which consists of *Stipa* spp. (30.1%), *A. chinense* (28.4%) and *C. microphylla* (6.7%) (Gao *et al*. 1995).

GENERAL HABITS AND ACTIVITY

1. Adaptation to Grasslands

Like other grassland dwellers such as the saiga, the Tibetan antelope, *Pantholops hodgsoni*, and the North American pronghorn antelope, *Antilocapra americana*, Mongolian gazelles can run very fast.   They can reach speeds of

60-65 km/hr, jump height up to two meters and lengths of 4-6 m with a maximum of 13 m (Лукашкин 1927). Mongolian gazelles find it difficult to run on ice or move in snow that is deeper than 20 cm (Bannikov 1954). Mongolian gazelles have keen eyesight but relatively poor senses of smell and hearing.

In order to obtain sufficient food, Mongolian gazelles must graze all day long during autumn and winter, whereas during summer they graze only from dawn to 10 : 00 or 11 : 00, and then again from 19 : 00 or 20 : 00 to dusk (Гептнер *et al.* 1961).

During summer, because sufficient water for their needs is contained in their green fodder, Mongolian gazelles are able to forage tens or even hundreds of kilometers away from open sources of freshwater.

2. Group Formation

Mongolian gazelles usually live in groups all year round, but in larger groups in winter than in summer. Group size increases from September to April in Russia (Гептнер *et al.* 1961). During summer, the largest groups consist of fewer than 100 individuals, and usually groups number about 20-30 individuals. From late August or early September onwards, group size increases to 60-80, or even to several hundreds in some cases. During the rutting period from late November to early January, group size further increases to reach 100-120 individuals. If snow falls, groups increase in size to several thousands or even 10,000 animals. These large groups begin to break apart during May and June (Bannikov 1954). During spring and autumn migrations, they form large groups, some as large as 80,000 animals (Lhagvasuren and Milner-Gulland 1997).

In Inner Mongolia, mixed groups were most common during spring (63.1%), autumn (51.0%) and winter (56.2%), however in summer female groups were most common (60.7%), and solitary individuals were common in male groups (Guan 1996). Before the rutting season from September to November, the male/female ratio is about 1.3, and males often form bachelor groups. These groups join to form larger groups during late November, then separate again from the beginning of the rutting season (Zhao 1963).

3. Society and Behavior

The social system of the Mongolian gazelle is not yet well understood, however it is known that they are polygynous with one male gathering on average 13 females into his harem (range 6-25, Lhagvasuren and Milner-Gulland 1997). In Russia, rutting begins in late November and continues until early January (Гептнер *et al.* 1961), whereas in Mongolia it begins during mid-November and continues until early February with the peak between mid-December and mid-January (Lhagvasuren and Milner-Gulland 1997). During the rutting season, males battle with each other though the fighting is not serious (Гептнер *et al.* 1961). Pregnant females close to parturition in spring move to open rolling countryside where it is easy for them to avoid disturbance (Bannikov 1954).

4. Migration

Mongolian gazelles migrate during winter. In the northern part of their range, this migration is from south to north, whereas in the southern part of their range it is from north to south or east (Bannikov 1954). Part of the southern population migrates from Mongolia to Inner Mongolia, and before the 1970s some migrated from Mongolia into Russia. Since the 1970s, however, and since the population has been so reduced, migration into Russia has not been reported. These migrations may have occurred because of reduced food availability in the center of the range.

During summer, gazelles travel widely over ranges of several hundred square kilometers, often moving more than 10 km in a day with distances increasing as forage deteriorates. During the parturition period, however, females stay in restricted areas (Гептнер *et al*. 1961). Gazelles do not migrate when food is abundant or when there is little snow, which indicates that their migrations may be adaptive to avoid food shortages and heavy snow (Bannikov 1954).

POPULATION ECOLOGY

During the 1940s, the population of Mongolian gazelles is estimated to have reached approximately 1.5 million, with one million in Mongolia and 500,000 in China. During the 1950s and 1960s in China, 200,000 gazelles were hunted each year (Xiao *et al*. 1982), and as a result of this over-hunting, combined with over-grazing and desertification, the population has decreased considerably during the last 40 years.

1. Age Estimation

On the basis of tooth eruption and wear, Zhao (1982) categorized Mongolian gazelles into seven age groups. Jiang *et al*. (1995) have determined the exact age of 224 gazelles by counting growth layers in teeth cementum and have shown that the accuracy of Zhao's (1982) method is 72.3%. Of the remainder of the samples, 69.4% were over- or under-estimated but within just one year. Therefore, for practical purposes in the field, Zhao's (1982) categories are useful.

2. Demography
a. Natality

Females become fertile at about 17–18 months of age (Гептнер *et al*. 1961). The gestation period is about six months, and parturition occurs during May and June in Russia (Гептнер *et al*. 1961), and from mid-June to mid-July in Mongolia (Lhagvasuren and Milner-Gulland 1997). The pregnancy rate of Inner Mongolian females older than 1.5 years is as high as 100% ($n=122$, Jiang *et al*. 1993), and over 90% in Russia (Bannikov 1954), although in two populations in Mongolia, it has sometimes been lower at 40% and 60–85% (Lhagvasuren and Milner-Gulland 1997). Fawns are usually born singly with twins only

occurring rarely (2.5-8.2%) in both Mongolia and in Russia (Bannikov 1954, Lhagvasuren and Milner-Gulland 1997).

The survival rate of fawns in their first summer reaches 80%. Because of the high rate of pregnancy and of fawn survival, the rate of increase of the population sometimes reaches 20-25% (Bannikov 1954). Zhao (1988) estimated that the annual rate of increase in Inner Mongolia was also considerable at about 27%.

b. Mortality

Predation, periodic epidemics and severe winters are the main causes of death of the Mongolian gazelle. The main predators are wolves, domestic dogs and steppe eagles, with manul, *Felis manul*, and red fox also able to catch newborn fawns. Wolves attack the gazelles during late winter and spring, particularly after rutting when males are exhausted and unable to run for long. In early summer, wolves attack pregnant females. According to Гептнер *et al.* (1961), birds such as kites and vultures sometimes attack young fawns.

Information on diseases contracted by Mongolian gazelles is limited, however, that diseases do seriously affect them is well documented. In 1974, for example, about 140,000 animals were killed in eastern Mongolia by an unknown disease, and since then similar outbreaks have occurred regularly, though fewer gazelles have died (Lhagvasuren and Milner-Gulland 1997). Captive Mongolian gazelles are known to suffer from "foot-and-mouth disease" (Оливков and Носова 1940, Цветаева 1941) and *Pasteurellosis* (Yuan 1991). Rotshil'd *et al.* (1988) showed that the high level of molybdenum in their onion diet can be a cause of *Pasteurella* infections.

Various parasites of the Mongolian gazelle have been found including: *Przevalskiana aenigmatica*, *Pharyngomyia dzerenae*, *Melophagus* spp. (Hippoboscidae), *Cysticercus tenuicollis*, *C. bovis*, *Eimeria* spp. (Coccidia) and warble flies (Hypodermatidae and Oestridae) (Колосов 1939, Мачульский 1941, Грунин 1950, Sugar 1981/1982, Minar *et al.* 1985).

In Mongolia, severe winters, occurring about once every seven years since 1932, have killed thousands of gazelles (Lhagvasuren and Milner-Gulland 1997), and heavy snows and food shortages were recognized by Bannikov (1954) as sometimes causing losses of one third or half of Mongolian gazelle populations.

c. Sex Ratio

In Inner Mongolia, the sex ratio varies from year to year, but is slightly biased towards males (M/F=1.1 in 1979, Xiao *et al.* 1982, and 1.3 in 1988, Jiang *et al.* 1993), whereas in Russia, Bannikov (1954) found it to be slightly biased to females (M/F=0.92). Subsequently, Lhagvasuren and Milner-Gulland (1997) have found ratios in Mongolia strongly biased to females (M/F=0.1-0.14 in autumn, 0.08 in winter, and 0.05 in summer).

d. Life Table

Jiang *et al.* (1993) estimated the age structure of the Inner Mongolian

gazelle population as consisting of fawns (0.5 year old, 39.7%), reproductive females (over 1.5 years old, 25.0%), and older animals (more than 4.5 years old, 12.7%).   Three mortality peaks were noted among 0.5 year olds (39.7%), 3.5 year olds (57.4%) and among those over 6.5 years old (100%).   This population was considered to be increasing because of the high proportion of young gazelles, the high rate of fecundity and the low mortality rate.

The oldest known-age individuals in an Inner Mongolian population of 1,026 animals were 7.5 year old males and 9.5 year old females (Jiang *et al*. 1995), making them much younger than other related ungulates.   For example, mountain goat, *Oreamnos americanus*, males reach 14 years of age and females 18 years (Cowan and McCrory 1970), chamois, *Rupricapra rupricapra*, have survived to about 22 years of age (Walker 1975) and male Japanese serow, *Capricornis crispus*, reach 20 years while females may live as long as 24 years (Miura and Tokida 1988).   Jiang (1990) considered that Mongolian gazelles live short lives partly because of quick teeth wearing.

The net reproductive rate (R_0) of a population in Inner Mongolia was 1.134 in 1979 and 0.864 in 1988, while the finite rate of increase (E) was 1.043 in 1979 and 0.954 in 1988 (Jiang *et al*. 1993).   The abrupt decrease in both R_0 and E between these years may have resulted from habitat deterioration such as desertification, overgrazing by livestock, and particularly from over-hunting and poaching.

As a result of over-hunting and poaching, gazelles have been exposed to shooting for longer periods.   Poachers shoot more rutting males just after the rut, and more pregnant and lactating females after the legal hunting period because they are easier to shoot.   As a consequence, the proportion of reproductive females in the total population dropped from 32.5% in 1979 to just 25.0% in 1988 (Jiang *et al*. 1993).   The reduction of pregnant and lactating females would result in a decrease in fecundity, and the reduction of reproductive males would result in unhealthy sex ratios.

CONSERVATION AND MANAGEMENT

Mongolian gazelles seem to be the Asian ecological equivalent of the pronghorn which is a member of the grassland ecosystem of North America. Both gazelles and pronghorns are highly adapted to northern dry grassland ecosystems, however, they differ because the grasslands where Mongolian gazelles live are unique, in as much as they are not truly natural but have been utilized by humans as grazing lands for thousands of years.   In the past, people maintained this ecosystem based on an understanding of suitable grazing levels from experience, and hence they avoided deterioration of the grasslands.   In other words, these grasslands are the historical product of a system of "sustainable use".   The Mongolian gazelle has long been a representative member of this managed grassland ecosystem.

The most significant natural mortality factors of the gazelles seem to be predation, periodic epidemics and severe weather, however, the factor causing

Mongolian gazelles to be endangered is human activity. These activities include over-hunting, poaching and deterioration of their grassland habitat resulting from the over-extension of cultivated lands and by over-grazing. The impact of poaching is extremely biased towards males because of their large body size and their horns making them particularly valuable. This leads to a strongly biased sex ratio and, as a consequence, reduces the fecundity of females. Lhagvasuren and Milner-Gulland (1997) have calculated that Mongolian poachers kill 80,000 animals each year, at least 80–85% of which are males. Poaching just after rutting and during the birth period reduces numbers of reproductive males and females. Over-hunting is also responsible for the decline of populations. The heavy harvest (100,000 each year) for meat for soldiers during the Second World War, and during severe winters after the war, probably resulted in the rapid decline of populations during the 1950s and 1960s (Sokolov *et al.* 1982). Deterioration of grasslands results in the disappearance of suitable habitats which reduce the carrying capacity of the environment. One major factor contributing to the decline of the population in western Mongolia is thought to be the construction of the Ulaanbaatar-Beijing railway at the end of the 1950s. This obstructed the gazelles' east-west migration routes (Lhagvasuren and Milner-Gulland 1997).

In Inner Mongolia the extent of the grasslands has been declining. Compared to 1965, the grassland area has decreased by 62,000 km², degraded grasslands have increased by 287,000 km², and total grass production has dropped by 30% (National Research Council 1992). As a result, the Mongolian gazelle is facing a dangerous situation. It was estimated that the Mongolian gazelle population before the 1940s was about 1,000,000 in Mongolia and 500,000 in China (Гептнер *et al.* 1961), but today just 300,000–500,000 remain in total.

Taking this situation into consideration, the Chinese government listed the Mongolian gazelle under its 1989 wildlife protection law as a Class II species for conservation. Under this law, nature reserves are to be established in the species' main distribution areas, and inspection of the condition of the habitat is to be made regularly. Construction projects that will degrade the habitat and trading of the gazelles and their parts are to be controlled. Hunting is prohibited and poaching may be prosecuted under criminal law.

In the Russian Federation's "Red Data Book", the Mongolian gazelle was listed as a "disappearing species". In Mongolia, hunting has been controlled since 1932, and in 1995 a new hunting law was introduced in order to control poaching (Lhagvasuren and Milner-Gulland 1997).

Establishment of hunting controls based on population ecology is necessary. Zhao (1988), who has taken into consideration the current system of hunting in China together with the ecology of the Mongolian gazelle, has recommended that the open season for hunting gazelles should be limited to the period from early November to the middle of December because body weight is greatest and the meat quality is at its best during this period. On the basis that the Mongolian gazelle's capacity for increase is high at about 25%, Zhao (1988) also recommended that hunting intensity should be limited to 19% of the total

population.   Because of its high reproductive capability, the Mongolian gazelle population would then be able to recover quite quickly despite continued hunting, once hunting and poaching are controlled.

Besides control of legal hunting, a reduction of poaching is also vitally important.   Both the Chinese and the Mongolian governments are trying to control poaching, but this is extremely difficult to carry it out in vast, remote steppe areas.   Consequently, nature conservation education is seen as crucially important in such areas.

Because of the rapid shrinkage of distribution and the reduction of population size, it is urgently necessary to establish reserves.   A steppe plain in the Matad-Somon area of Mongolia (Fig. 1) is recommended as a reserve (Sokolov *et al*. 1982, Lushchekina *et al*. 1985, 1986).   The first national park of Mongolia, the Eastern Steppe National Park, was established in 1995 to conserve the Mongolian gazelle.   More reserves are needed in China.   The reintroductions have been done in 1978 and 1988-1990 in Mongolia and small populations survived in Dzavkhan, Gobi-Altai and Uverkhangai maybe because of the reintroductions.   Trials of captive breeding in the reserves and transplantation should be considered.   Studies on epidemics, migration routes, genetic structure *etc*. are also needed.   Since Mongolian gazelles migrate between Mongolia and China, consistent plans for management, conservation and cooperative activities between the countries are necessary.   Grassland productivity should be improved based on both agricultural and ecological sciences.   The traditional grazing system of Mongolian people should be also reconsidered.

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Short Communication

Seasonal change in the testis size of the Japanese giant flying squirrel, *Petaurista leucogenys*

Takeo KAWAMICHI

Department of Biology, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka, Japan 558-8585
Fax. +81-75-551-3936, e-mail, pika@sci.osaka-cu.ac.jp

In seasonally breeding male mammals, the testes generally regress completely during the non-breeding season.   This is followed by a "reawakening" of the regressive testes in a process known as recrudescence (Nalbandov 1976).   For the accurate estimation of testis size throughout the year, many individuals have to be killed at different seasons (*e.g.,* 116 male flying squirrels, *Petaurista petaurista*, by Lee *et al*. 1993), however, such study methods cannot be applied to protected animals such as the Japanese giant flying squirrel, *P. leucogenys*.   I have adopted an alternative method which involves no harm to study animals. This involves, instead, estimating testis size during natural observations of squirrels above me in trees.   Despite this being only a rough estimation, it is easy to perform, and the data obtained are useful in understanding the mating system and reproductive cycle of *P. leucogenys*.

From my behavioral and ecological studies of *P. leucogenys*, I have already confirmed the existence of two mating seasons, the first from mid-November to mid-January, and the second from mid-May to mid-June (Kawamichi *et al*. 1987).   The two intervals between these mating seasons are in different seasons, winter and summer.   There is no information, however, on the seasonal changes in the testis size of this species.   In the genus *Petaurista*, the details of seasonal change in testis weight are known only for *P. petaurista* in Taiwan (Lee *et al*. 1993).   In this paper, therefore, I describe visual estimates of testis size of wild *P. leucogenys*, and discuss seasonal changes in testis size in relation to the species' biannual mating seasons.

MATERIALS AND METHODS

The study area consists of 0.65 km² (65 ha) in a temperate mixed forest of deciduous and coniferous trees.   It is situated at 34°41'N, 135°50'E, at an elevation of 98-150 m, adjacent to Nara City in central Japan (see Kawamichi 1997a). Snowfalls occur occasionally in winter, but snow-cover lasts only a few days.

Observations were conducted during 977 nights from 1983 to 1990.   *P. leucogenys* were located by walking at random through the forest at night using a 9-volt searchlight.   Nikon zoom binoculars (8-16×, Tokyo) were used to identify all resident squirrels by the scars on their ears and by the details of

their pelage.   The testes of known individual males were observed, illustrated, and classified into four size categories : 1) full-size, 2) 2/3 to 3/4, 3) 1/3 to 1/2, and 4) complete regression.

RESULTS

A total of 667 estimates of testis size was made for 52 resident adult males. These males were observed for up to six years, and ten were observed continuously from before they became sexually mature.   Testis condition was determined bimonthly (see Fig. 1).

During the two mating seasons, from mid-November to mid-January and from mid-May to mid-June (Kawamichi *et al*. 1987), more than 80% of adult male *P. leucogenys* had full-sized testes (Fig. 1).

Each year testes regressed soon after the May/June mating season (Fig. 1), and by July no males had full-sized testes, and 55% already had fully regressed testes.   Given that in the first half of June 81% of adult males still had full-sized testes, the speed of regression during late June was considerable, and the difference between the proportion of males with full-sized testes during the second half of June, and the first half of July was statistically significant (Fisher's exact probability test, $p=0.0007$).

During the first and second halves of July, testes assessed as "small" (1/3 to 3/4 size) included both those regressing and those already redeveloping. From the first half of August, however, all small testes were in the process of
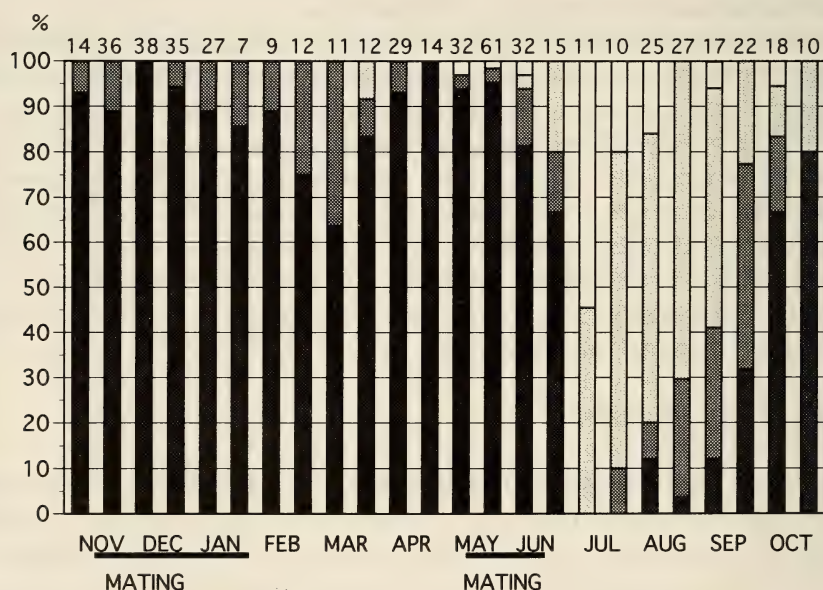
Fig. 1.   Bimonthly changes in the testis size (estimated visually) of wild, adult male *Petaurista leucogenys*.   Figures on the top are sample sizes.   The gradation from dark to white bars indicates testis size : full-size, 2/3 to 3/4, 1/3 to 1/2, and complete regression, in that order. Horizontal bars indicate the mating seasons.

redevelopment.   The proportion of males with full-sized testes reached 80% again in the second half of October, one month before the next mating season.

During the second half of February the proportion of males with full-sized testes decreased to 75%, and during the first half of March this further decreased to 64%.   The remaining males had 2/3 to 3/4 sized testes during these periods.   In only one 19-month-old male, did the testes regress from March right through the May/June mating season.

The complete process of testicular regression through to redevelopment was observed 34 times in 24 different males.   The period from the beginning of regression to the early stage of redevelopment ranged from 42 to 57 days and averaged 47 days (±2.6, *SE* ; $n=5$).   There was, however, great individual variation.   The earliest case of regression was found on 4 June, while one male still had full-sized testes until 22 June.   Redeveloped, full-sized testes were first observed on 8 September, although one male still had almost fully regressed testes on 14 September.

Of 12 males observed on 24, 30, and 31 July, only three had testes which were beginning to redevelop, but within the first week of August, the early stages of testicular redevelopment were recognized in 10 out of the 12 males. Although there were not enough data in late June, the main period of regression was assumed to be from late June to late July, and the period of redevelopment was assumed to be from late July through October.

DISCUSSION

During summer, there was much variation in testis size in the male population.   Some adult males still had regressive testes at the same time that others already had redeveloping testes.   Yearling males born in the early spring of the previous year, begin to develop visible testes for the first time during summer (Kawamichi 1997b).   Thus, only year-round observations of males from when they are still sexually immature onward will reveal the complexity of change in testis size in the male population during summer.

Lee *et al*. (1993) found that of 116 male *P. petaurista* collected in Taiwan, the weights of testes and epididymides showed the same two peaks, from March to June, and from October to November.   Although these peak seasons were different from those of *P. leucogenys*, the presence of two active seasons, separated by an interval of a few months, is similar to that of the *P. leucogenys* described in this study.

Lee *et al*. (1993) found that in *P. petaurista*, spermatogenesis degenerated during the periods from June to August and from December to March, that is, during the intervals separating the two active seasons.   Complete regression of testes during summer has also been observed in a captive male *P. leucogenys* (Kawamichi per. obs.).   Although anatomical analyses of testes during the period from February to March are required for confirmation, it appears that 64% of adult males had full-sized testes, and the remaining 36% had testes of 2/3 to 3/4 size in the first half of March (Fig. 1), whereas no adults had full-sized

testes in July.   This suggests that the small size of testes in 36% of adult males was due to the contraction of the scrotum at low temperature (mean minimum air temperatures obtained from Nara City Meteorological Station were $-0.5°C$ in February and $1.6°C$ in March).   Further study is required to clarify whether testicular regression really occurs in winter in all parts of the male population or not.

The exact interval between matings during winter, that is, from the last mating on 29 January to the earliest one on 12 May, was 102 days.   This period was 51 days shorter than the interval between the last mating on 16 June and the earliest one on 17 November (153 days) during summer (Kawamichi unpubl. data).   During the summer non-mating period, the steady increase in the proportion of males with full-sized testes covered four months (Fig. 1).   The mean duration from the beginning of regression to the early stage of redevelopment, was 47 days, although there was a great deal of individual variation. These facts suggest that the interval of 102 days during winter may not be sufficient for functional testicular redevelopment in the male population.

The testes regressed rapidly in June, during or soon after the May/June mating season.   Therefore, if females failed to become pregnant, they could not mate again until the next mating season from mid-November onward. Testicular regression may be related to the fact that the May/June mating season is one month shorter than the November to January mating season.

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Errata (Mammal Study, Vol. 22 [1/2])

Cover back page, line 5, Ryosuke Nakata should read Keisuke Nakata

page 2, line 43, *Taireria* should read *Theileria*

page 3, line 1, *Taireriosis* should read *Theikeriosis*

page 3, line 4, paras-italogical should read paras-itological

page 3, by the Australian government should read by the Australian-Japan Foundation, the Australian government

page 3, line 9, Southerncross should read Southern Cross

page 3, line 24, Mori should Mōri

page 4, line 10, *Tikusnema javaense* n, gen. should read *Tikusnema javenense* n. gen

page 4, line 13, Gene's should read Geně's. Mori should Mōri

page 4, line 14, 16 : 63−275 should read 16 : 263−275

page 4 , line 22, Ohdachi, S., R. Masuda, H. Abe, J. Adachi, N. E. Dokuchaev, V. Hasegawa, H., S. Shiraishi and Rochman. 1992. *Tikusnema javaense* n, gen., n. sp. (Nematoda : Acuarioidea) and other nematodes from Rattus argentiventer collected in West Java, Indonesia. J. Parasit. 78 : 800−804. should read Ohdachi, S., R. Masuda, H. Abe, J. Adachi, N. E. Dokuchaev, V. Haukisalmi,and M. C. Yoshida. 1997. Phylogeny of Eurasian soricine shrews (Insectivora, Mammalia) inferred from the mitochondrial cyto-chrome b gene sequences. Zoological Science 14 : 527−532

page 4, line 32, Yoshinaga, Y. and Shiraishi should read Yoshinaga, Y., T. Okayama, W. Ohno and S. Shiraishi

page 4, line 34, Mori should read Mōri

page 43, Ando, A. and S. Shiraishi. 1997. Eye lens weight for age determination in Smith's red-backed vole, *Eothenomys smithii*. Mammal Study 22 : xx – xx. should read Ando, A. and S. Shiraishi : Eye lens weight for age determination in Smith's red-backed vole, *Eothenomys smithii* : Mammal Study 22 : 45−52

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Eisenberg, J. F. 1981. The Mammalian Radiations. Univ. of Chicago Press, Chicago, 610 pp.

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Obara, Y. 1991. Karyosystematics of the mustelid carnivores of Japan. Honyurui Kagaku [Mammalian Science] 30 : 197−220 (in Japanese with English abstract).

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Mammal Study

Contents

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Postnatal development of the neuromuscular junction of the masseter muscles in the Japanese field vole, *Microtus montebelli*

Katsumi Sugasawa[1], Kōichi Andō[2] and Takayuki Mōri[1]*

[1] *Zoological Laboratory, Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812-0053, Japan*
[2] *Biological Laboratory, Department of Regional Culture, Faculty of International Studies of Culture, Kyushu Sangyo University, Fukuoka 813-0004, Japan*
* To whom correspondence should be addressed
Fax. +81-92-642-2804, e-mail : sugar@agr.kyushu-u.ac.jp

Abstract. The developmental pattern of the neuromuscular junction (NMJ) in masseter muscles of the Japanese field vole, *Microtus montebelli*, was investigated using acetylcholinesterase (AChE) staining and electron microscopy. At birth, intense AChE activity limited to the site of the NMJ where many axon terminals with the cholinergic nature were converging was observed, indicating that cholinergic neuronal activity in the vole masseter muscle begins at this stage. The major morphogenesis of the NMJ such as : AChE staining reaction, concentration of myonuclei at the subneural site, elimination of the axon terminals, formation of the myeline sheath in the intramuscular axons, and the appearance of numerous junctional folds in the postsynaptic membrane was accelerated after postnatal day five, and amended dramatically at day ten with the maturation time of these NMJ components at around day fifteen. From the combination of the present and previous studies, it is clear that both AChE reaction and neuronal structures alter considerably at a time when structural and functional improvements give rise dramatically to muscle fibers. This must be considered in relation to the critical role of the neuronal influence on the differentiation and maturation of the vole masseter muscles that are required for the accomplishment of its own characteristic herbivorous food habits.

Key words : acetylcholinesterase, masseter muscle, neuromuscular junction, postnatal development, ultrastructure.

Among the masticatory muscles of the rodents, the masseter muscle is the largest, and is hence regarded as the most functional muscle during biting. Our recent ontogenical study of a particularly successful herbivorous rodent, the Japanese field vole, *Microtus montebelli* (Sugasawa and Mōri 1997), revealed that vole masseter muscles differentiate abruptly after birth and mature fully before weaning. This developmental pattern of the masseters is distinctly different from that of the masseters of either the rat, *Rattus* spp. or the mouse, *Mus* spp., in which they remain immature even at postnatal day

(PND)23, when the young are weaned from their mothers (Maeda *et al.* 1981, Hurov *et al.* 1992, Miyata *et al.* 1996).

It is well established in both the rat and the mouse, that neuronal actions and factors are significantly involved in the differentiation of developing skeletal muscles in various parts of the body (Ridge 1989, Hall and Sanes 1993, Grinnell 1995). Nevertheless, little is known about the neuromuscular interaction in the ontogenical process of the masseter muscle. Therefore, as the first step towards an understanding of the neuronal influence on the development of the rodent masseter muscle, we examined the developmental pattern of the neuromuscular junction (NMJ) in the vole masseter muscles from birth to PND 15, using histochemistry for acetylcholinesterase (AChE), the enzyme hydrolyzing acetylcholine (ACh) and electron microscopy.

MATERIALS AND METHODS

The Japanese field voles used for this study were obtained from our laboratory colony which originated from wild voles live-trapped in Fukuoka Prefecture. They were kept in cages in an environment-controlled room ($23 \pm 1°C$, LD 14 : 10). All the animals were given a herbivorous diet (ZF, Oriental Yeast Co., Ltd., Tokyo), a commercial mouse diet (NMF, Oriental Yeast Co., Ltd., Tokyo) and water *ad libitum*. Twenty four newborn voles of both sexes, which were kept with their mothers in cages, were used in this study. The day of birth (day 21 or 22 of gestation) was regarded as postnatal day 0 (PND 0). The animals were divided by age into the following four groups : PND 0, PND 5, PND 10, and PND 15. For each of the four groups, three individuals were used for AChE histochemistry and electron microscopy.

1. Histochemistry for AChE

The voles were anesthetized with ethyl ether then perfused through the left ventricle with Ringer's solution, followed by 30 ml of ice-cold 4% buffered formaldehyde. The masseter muscles were carefully dissected from the jaws, then postfixed with the same fixative for one hour. They were washed thoroughly with 0.1 M phosphate buffer (PB, pH 7.4), and then immersed sequentially in PB containing 10% and 20% sucrose for two days each at 4°C. For sectioning, the materials were quickly frozen in isopentane chilled with dry ice and sectioned at a thickness of 20 μm in a cryostat. To detect AChE activity, sections were maintained in substrate (acetylcholine iodide, Sigma Chemical, USA), free Karnovsky's medium (Karnovsky and Roots 1964) for 30 min at 4°C, and incubated in the complete medium containing 2×10^{-4} M tetraisopropyl pyrophosphoramide (Sigma Chemical, USA) as an inhibitor of non-specific cholinesterase for one hour at 20°C. These procedures have been described in detail by Andō (1981).

2. Electron microscopy

In order to avoid excessive muscular contraction during direct fixation,

once the voles were fully anesthetized they were decapitated, and the heads were first immersed for 20 min in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate (SC) at pH 7.2. Subsequently, the masseter muscles were dissected out from the jaw in the same fixative, and postfixed for two hours. The materials were washed briefly in 0.1 M SC, and fixed for a further two hours in 1% osmium tetroxide buffered with 0.1 M SC. The tissues were dehydrated in an ethanol series and embedded in Epon 812. Thin sections ($\sim$60 nm) were cut on a Porter-Blum MT-1 microtome using a glass knife, and doubly stained with lead and uranyl acetate before examination in an Hitachi-H600A electron microscope (75 kV).

RESULTS

1. AChE-activity

In all of the voles examined at birth, AChE activity was observed to be limited to the center of the muscle fibers where the NMJ is formed. The muscular areas stained specifically with AChE were shaped like a button with a diameter of about 3.5 μm, and showed a linear profile in the transverse direction (Fig. 1a). The staining reaction at this site did not change signifi-
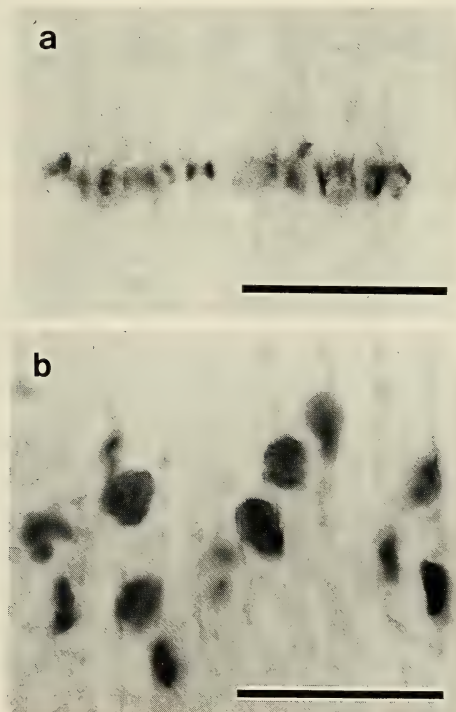
Fig. 1. AChE reaction of the masseter muscle in the vole at birth (a) and on PND 15 (b). Bar : 50 μm.

cantly from PND 0 to PND 5, however, by PND 10, the AChE-positive area had increased to about three times the diameter observed at birth with a rise in the activity of this particular enzyme.   The AChE-positive area enlarged further after PND 10, reaching approximately five times the area observed at birth on PND 15.   The enzyme activity also became more prominent (Fig. 1b).   No detectable difference in the AChE staining properties of the NMJ was observed between the neonates at PND 15 and maternal voles at six months of age.

2. Electron microscopic observations

At birth, many axon terminals covered with Schwann's cells converged at the site of the NMJ, (Fig. 2a).   These axon terminals did not contain the cytoskeletal or membranous components indicative of growth cones, but accumulated with small clear vesicles (about 50 nm in diameter) indicating their cholinergic nature (Fig. 2b).   At this stage, although the myeline sheath was not yet formed, most of the intramuscular axons were already encircled by Schwann's cells (Fig. 2c).   The basal lamina extended over the synaptic cleft in the muscle fibers.   On the one hand, no junctional folds or subneural nuclei were seen, nor was an accumulation of mitochondria noted in the soleplate region.   On the other hand, the subneural muscle plasma membrane was undercoated with an electron-dense amorphous material, showing a profile similar to the postsynaptic membrane in adults (Fig. 2b).

No appreciable difference in the number of axon terminals in the NMJ was observed between birth and PND 5, however each of the intramuscular axons was surrounded by a thin myeline sheath by PND 5 (Fig. 3a, b, c).   After PND 5, some structural specializations in the muscle fibers were observed in the soleplate region.   Numerous myonuclei were concentrated at the subneural site, forming the subneural nucleus (Fig. 3a).   In parallel with this, the muscle plasma membrane began to invaginate and form junctional folds (Fig. 3c).

By PND 10, the number of axon terminals had decreased markedly (Fig. 4a), and the myeline sheaths of the intramuscular axons became much thicker (Fig. 4b).   At this stage, the junctional folds increased greatly in number and grew taller owing to the frequent occurrence of deep invaginations of the plasma membrane.   The accumulation of mitochondria, though not so prominent, was also observed in the soleplate regions (Fig. 4a).

By PND 15, the NMJ had only one axon terminal that was filled with small clear vesicles and small mitochondria, which was also furnished with numerous, regularly arranged junctional folds that were very tall and showing structural properties similar to those seen in adult voles (Fig. 5a, b).

DISCUSSION

The present study has shown for the first time an age-related change in AChE-activity and in the neuronal elements in the NMJ of the vole masseter muscle.   At birth, high AChE activity was localized specifically at the NMJ, where a number of axon terminals with small clear vesicles, typical of a
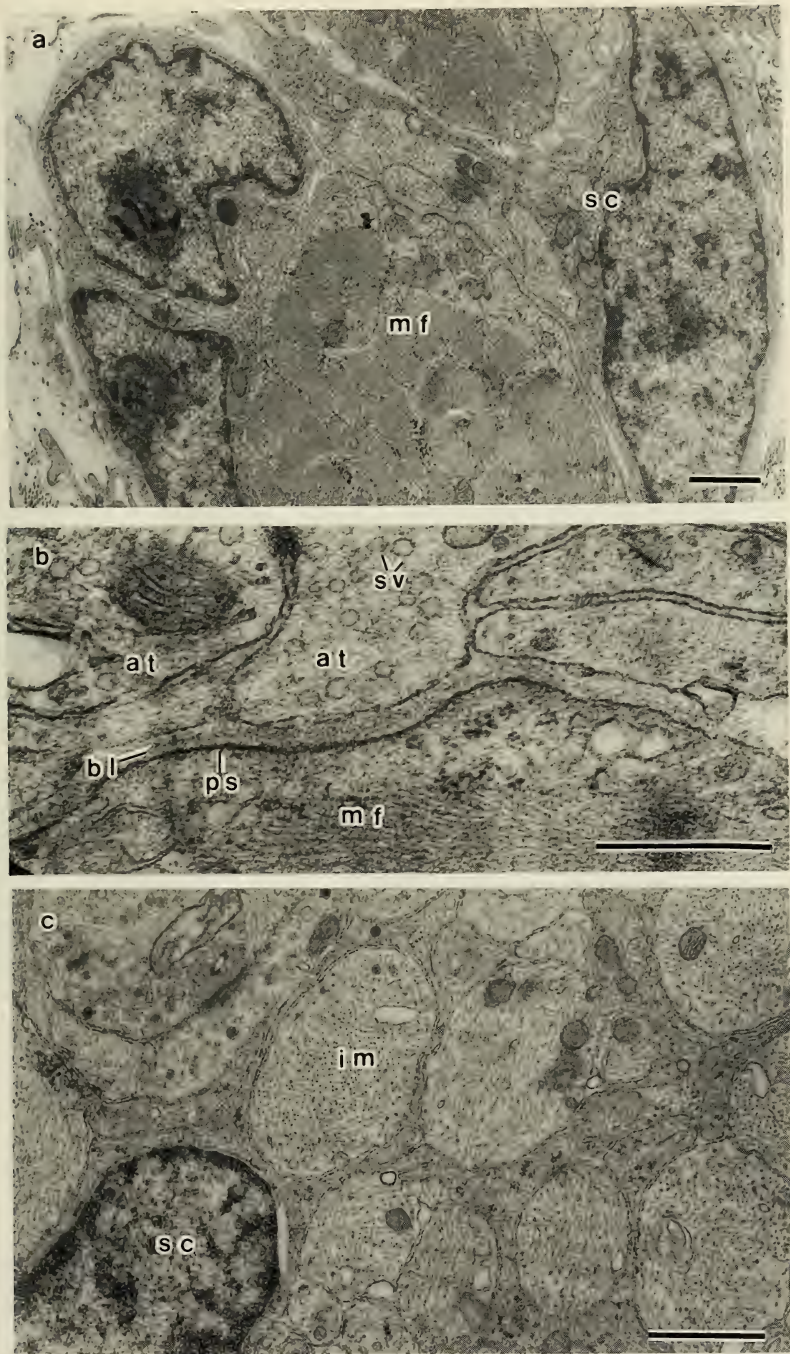
Fig. 2.   Electron micrographs of the NMJ (a, b) and intramuscular axons (c) in the vole masseter muscle at birth.   at : axon terminal, bl : basal lamina, im : intramuscular axon, mf : myofiber, ps : postsynaptic membrane, sc = Schwann's cell, sv : small clear vesicle. Bar : 1 μm (a, c), 0.5 μm (b).
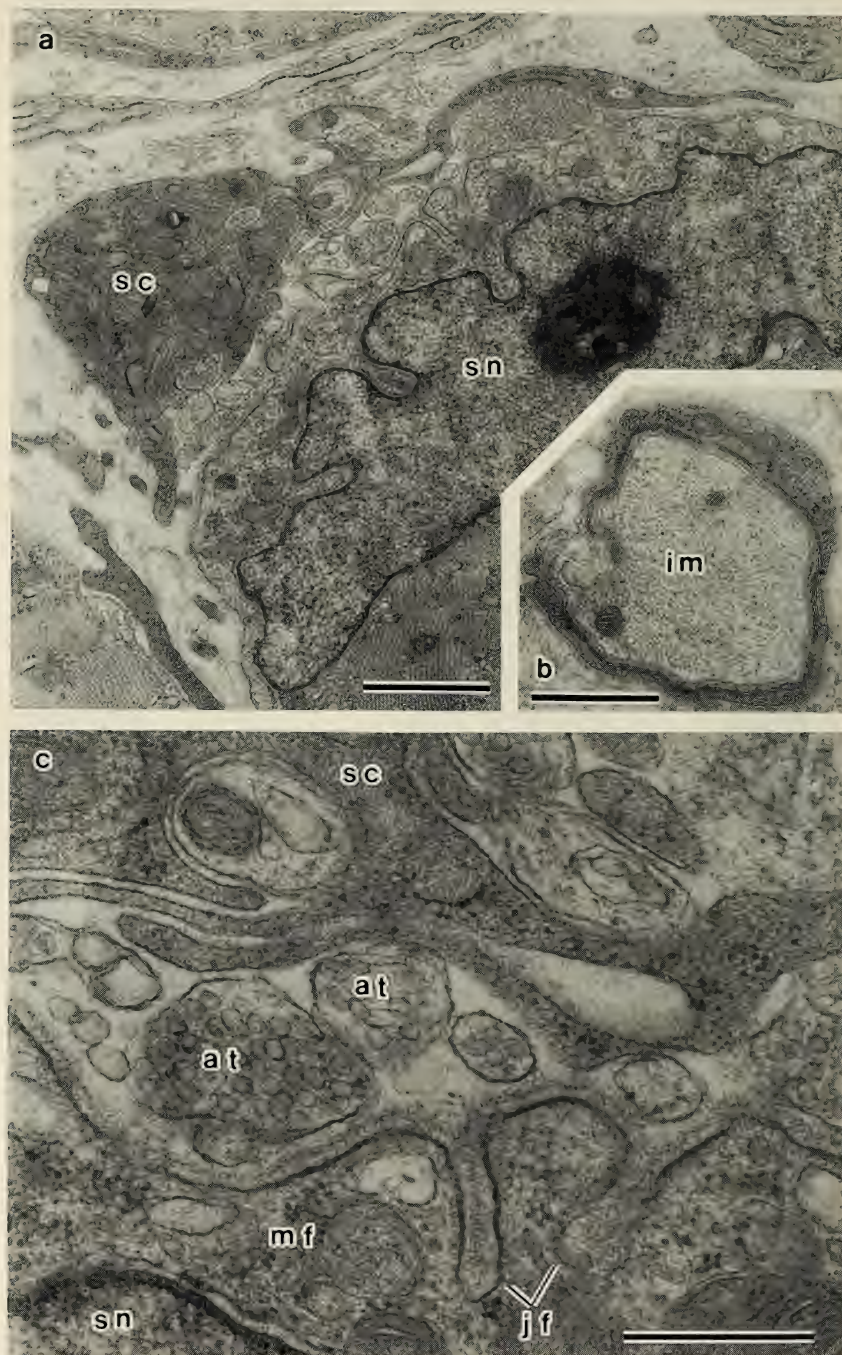
Fig. 3.   Electron micrographs of the NMJ (a, c) and an intramuscular axon (b) in the vole masseter muscle at day 5.   at : axon terminal, im : intramuscular axon, jf : junctional fold, mf : myofiber, sc : Schwann's cell, sn : subneural nucleus.   Bar : 1 μm (a, b), 0.5 μm (c).
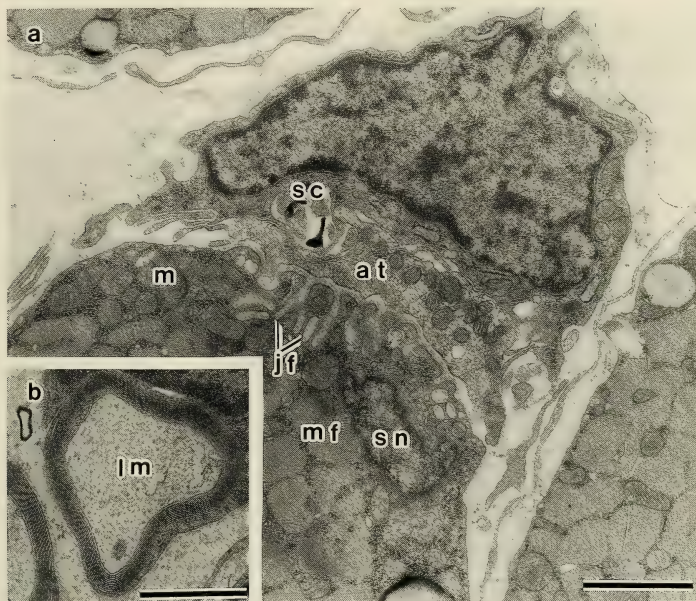
Fig. 4. Electron micrographs of the NMJ (a) and an intramuscular axon (b) in the vole masseter muscle at day 10. at : axon terminal, im : intramuscular axon, jf : junctional fold, m : mitochondrion, mf : myofiber, sc : Schwann's cell, sn : subneural nucleus. Bar=1 μm.

cholinergic nature, were concentrated. In addition, the muscle plasma membrane was found to be similar in basic structure to the postsynaptic membrane of adults. These results seem to suggest that neuronal action, provoked by cholinergic transmission (release and hydrolysis of ACh), is operational in the vole masseter muscles at birth, whereas studies of the hind leg muscles of the domestic fowl have indicated that AChE activity, specific for the NMJ, appears some days after the onset of synaptic transmission has been provided (Grinnell 1994).

The present study has further shown that : the major morphogenesis of the NMJ, which is represented by concentration of myonuclei at the subneural site, elimination of the axon terminals, formation of the myeline sheath in the intramuscular axons, and the appearance of numerous junctional folds in the post synaptic membrane, are all accelerated after PND 5 and amended dramatically on PND 10. Likewise, AChE-staining areas enlarged greatly, with a marked rise in enzyme activity, at a stage of development coinciding with the time (PND 10) when young voles start to take solid food, and when the muscle fibers begin to change from an undifferentiated condition into their own specific (fast twitch oxidative) fiber type and to display a potent contractive ability (Sugasawa and Mōri 1997).

By PND 15, the AChE-staining reaction and the neuronal structures in the NMJ attained levels equivalent to the mature pattern observed in six months

old adult voles.   This stage coincides with the expression of strong oxidative enzyme activity in the muscle fibers and the commencement of their sustained contraction in adulthood (Sugasawa and Mōri 1997).

As described above, in the ontogenical process of the vole masseter muscle, both AChE activity and the neuronal structures in the NMJ changed consider-ably at a time when structural and physiological improvements give rise dramatically to muscle fibers with a synchronous maturation of NMJ compo-
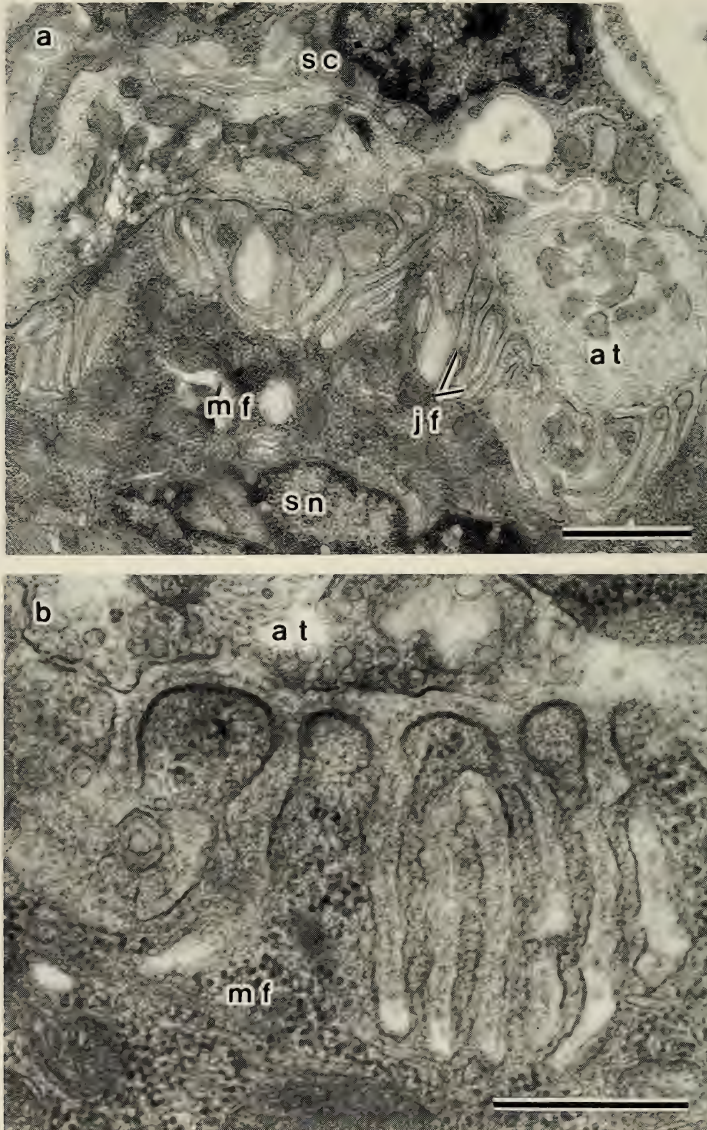
Fig. 5.   Electron micrographs of the NMJ in the vole masseter muscle at day 15 (A, B).   at : axon terminal, jf : junctional fold, m : mitochondrion, mf : myofiber, sc : Schwann's cell, sn : subneural nucleus.   Bar=1 μm (a), 0.5 μm (b).

nents. Thus, there is good correlation between age-related changes in neuronal and in muscular elements of the vole masseter NMJ. Although no direct evidence, as to which factors and mechanisms participate in the development of the masseter muscles, was provided by the present study, these findings seem to indicate the great significance of the neuromuscular interactions responsible for a chain of ontogenical events on this masticatory muscle. Based on evidence of the functional involvement of cholinergic transmission, particularly its trophic effect, in the starting and advance of muscle ontogeny (Grinnell 1994), such neuronal influence might play a critical role in the differentiation, during each developmental stage, of the vole masseter muscles. This may be closely related to species-specific requirements, for the structural and physiological maturation of this masticatory muscle, which are essential for the accomplishment of the herbivorous food habits so characteristic of the voles.

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Microsatellite DNA variations of the sika deer, *Cervus nippon,* in Hokkaido and Chiba

Junko NAGATA[1], Ryuichi MASUDA[2], Koichi KAJI[3], Keiji OCHIAI[4], Masahiko ASADA[4] and Michihiro C. YOSHIDA[2]

[1] *Wildlife Management Laboratory, Forestry and Forest Products Research Institute, Ibaraki 305-8687, Japan*
Fax. +81-298-73-1543, e-mail. junbe@ffpri.affrc.go.jp
[2] *Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*
[3] *Hokkaido Institute of Environmental Sciences, Sapporo 060-0819, Japan*
[4] *Natural History Museum and Institute, Chiba, Chiba 260-0852, Japan*

Abstract. To study genetic diversity of populations of the sika deer, *Cervus nippon*, in Hokkaido, polymorphism of three microsatellite loci (OarFCB193, BOVIRBP and INRA040) were examined and compared with that of the population of Chiba Prefecture in the Japanese main island (Honshu). The microsatellite genotype frequency significantly well agreed with the Hardy-Weinberg expectation at each locus of each population except for INRA040 of the Chiba population. Average observed heterozygosity ($Ho = 0.21 \pm 0.11$) of the Hokkaido population was relatively smaller than that of the Chiba population ($Ho = 0.23 \pm 0.09$). Moreover, observed heterozygosities of OarFCB193 and BOVIRBP of the Hokkaido population were lower than that of the Chiba population, and the number of alleles observed at each locus was smaller in the Hokkaido population than in the Chiba population. These results indicate a lower genetic diversity in the Hokkaido population, resulting from their historical bottleneck(s) previously reported. The present study provides information of useful microsatellite markers and gives an insight for better understanding population genetics of the Japanese sika deer.

Key words : bottleneck, *Cervus nippon*, heterozygosity, microsatellite DNA, sika deer.

The Japanese sika deer, *Cervus nippon*, is a large herbivore which is distributed through the Japanese islands. Overpopulation of the sika deer occurring on small islands such as Kinkazan of Miyagi Prefecture, Goto of Nagasaki Prefecture, and Nakanoshima of Hokkaido has caused not only severe damage to island forests, but also suffered from their own malnutrition (Ohtaishi 1986, Kaji *et al.* 1988, Whitehead 1993, Takatsuki 1994, Kaji 1995).

In Hokkaido, which is the northernmost island of Japan, the sika deer population suffered from a crash due to heavy snow falls especially during the winter of the year 1879 in the Meiji era of the Japanese history (Inukai 1952) and then most of local populations were extinct (Hokkaido Government 1986). After then, because Hokkaido Government controlled harvest of the sika deer

for conservation, the population remarkably recovered from a very small number during the last 40 years (Kaji *et al.* 1988, Hokkaido Government 1986, 1994, Kaji 1995,).   To date, the sika deer has been populated most densely in the central and eastern areas of Hokkaido.   Between 1980 and 1993, the annual harvests reported by regular hunters increased from approximately 3,500 to 26,000 animals in Hokkaido (Kaji 1995).   Recently, increasing of damage to agricultural crops, plantations, natural forests and traffic accidents brought by the sika deer has become the severest social problems in Hokkaido.   Therefore, it is urgently needed to control and manage the sika deer population (Hokkaido Government 1994).

On the other hand the Hokkaido population of the sika deer is likely to have reduced a level of genetic variation through the bottleneck(s) despite a large population size, because genetic drift by bottleneck is reported to generally drive neutral genetic variability to a very low level (Nei *et al.* 1975, Chakraborty and Nei 1977, Viard *et al.* 1996,).   Such a decrease of genetic variability can lead to inbreeding depression, loss of evolutionary flexibility and greater susceptibility to some disease resulting in possible extinction of the population (O'Brien and Evermann 1988).   The harvest without considering genetic diversity could bring depression of viable potential in the population. However, because scientifically definitive data on the sika deer genetic diversity in Hokkaido was lacking, it was very necessary and urgent to investigate genetic variation in the population for control and conservation.

Microsatellite DNA is known as a highly polymorphic and neutral Mendelian marker of nuclear genome (Queller *et al.* 1993).   This kind of DNA region includes tandem repeats of 1–5 base units, and alleles are defined as the number of polymorphic repeats.   Such hypervariable numbers of repeats are used extensively for identifying individuals and paternity as well as for population genetic studies (Queller *et al.* 1993, Abernethy 1994, Pépin *et al.* 1995, Viard *et al.* 1996).

The objective of the present study is to clarify genetic variation of such microsatellites in the sika deer population of Hokkaido and then to understand population diversity for conservation and management.   We here present alleles and their frequencies obtained at their microsatellite loci and discuss genetic diversity and characteristics of the Hokkaido population and Chiba population in the middle of Honshu, the mainland of Japan.

MATERIALS AND METHODS

1. Animal collections and DNA extraction

A total of 110 sika deer samples (blood, muscle or liver tissues) were obtained in Hokkaido from 1991 to 1996 in cooperation with scientists, town offices and hunters.   Especially, Nature Conservation Department of Hokkaido Government kindly supported systematic sampling collection.   Sampling was done as widely as possible in various areas of Hokkaido, and as to avoid an inclination of areas.   No animals with known familial relationships were

used for analysis.　Thirteen liver samples of the Chiba population were used as a control population.

　　From whole blood or tissues, total DNA was extracted using the phenol/ proteinase K/sodium dodecyl sulfate (SDS) method of Sambrook *et al.* (1989) with a slight modification (Masuda and Yoshida 1994).　Using a small glass homogenizer, a small piece of tissue (approximately $2 \times 2 \times 2$ mm) was homogenized in 500 μl of STE buffer (100 M NaCl/10 mM Tris, pH7.5/1 mM EDTA) containing a final concentration of 0.5% SDS and 5 μg/ml of proteinase K. The homogenate was incubated at 37°C overnight.　In case of blood, 100 μl was treated in 400 μl of STE buffer.　DNA was purified by extracting at least twice with the equal volume of phenol/chloroform (1 : 1) and once with chloroform/ isoamyl alcohol (24 : 1).　These procedures were done in 1.5 ml microcentrifuge tubes.　Extract without any animal tissue was used as a negative control in the following polymerase chain reaction (PCR) amplification.

2. Microsatellite analysis

　　Microsatellite loci established from the bovine or sheep were used for analysis : BOVIRBP, OarFCB193 (Abernethy 1994), and INRA040 (Vaiman *et al.* 1994) (Table 1).　PCR amplification of microsatellites was performed using a PCR reagent kit (GibcoBRL) according to the manufacturer's instruction. One microliter of the DNA extract was subjected to PCR amplification in a total volume of 25 μl of a reaction mixture including 20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at 0.2 mM, 1.25 U of Taq DNA polymerase and each Texas Red-labeled primer at 0.5 μM.　After the first step of denaturing at 94°C for 5 min, 26-30 cycles of amplification were realized (94°C for 15 sec, 55°C for 15 sec, 72°C for 20 sec) followed by reaction completion at 72°C for 10 min.　PCR products (0.2-1.0 μl) were then loaded with 2 μl of the loading buffer on an 6-8% Long Ranger gel (FMC BioProducts) and run for 10 hours using DNA Sequencer SQ-5500 (Hitachi).

　　Molecular sizes of alleles at each locus were identified from difference of electrophoretic mobility of PCR product bands using the computer program FRAGLYS ver. 2 (Hitachi).　To assess genetic variability within each population, expected heterozygosity (*he*) was calculated for each locus using the

Table 1.　PCR primer sequences for microsatellite DNA analysis in the present study.

Microsatellite locus	Primer sequence (5′-3′)	References
BOVIRBP	TGTATGATCACCTTCTATGCTTC GCTTTAGGTAATCATCAGATAGC	Abernethy (1994)
OarFCB193	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	Abernethy (1994)
INRA040	TCAGTCTGGAGGAGAGAAAAC CTCTGCCCTGGGGATGATTG	Vaiman *et al.* (1994)

formula $he = 1 - \Sigma X_i^2$, where X_i is the ith allele frequency of the locus in the population. An average of expected heterozygosity (*He*) was calculated using the formula $He = \Sigma he_j / r$, where he_j is heterozygosity of the jth locus and r is the number of analyzed loci. Microsatellites genotype frequencies were tested against the Hardy-Weinberg's expectation for each locus in the population using the computer program Arlequin ver. 1.0 (Schneider *et al.* 1997). Genetic differentiation between populations was estimated with Weir and Cockerham's (1984) *Fst* value. The significance of *Fst* value was tested using the permutation procedure in Arlequin ver. 1.0.

RESULTS

At two microsatellite loci (OarFCB193 and BOVIRBP), clear bands of 100 –150 base-pairs (bp) were identified as alleles. The INRA040 locus provided prominent bands (188-240 bp) with a few weak bands (Table 2). Animals from the Hokkaido ($n = 93$ for OarFCB193, $n = 108$ for BOVIRBP, and $n = 100$ for INRA040) and the Chiba ($n = 13$ for all loci) populations were analyzed to estimate allele frequencies for each microsatellite locus. At OarFCB193 locus, two alleles in Hokkaido and four alleles in Chiba were found. At INRA040 locus, two alleles in Hokkaido and five alleles in Chiba were found (Table 2),

Table 2. Microsatellite variation in the Hokkaido and Chiba populations.

	Locus		Hokkaido	Chiba
OarFCB193	No. individuals		93	13
	Allele* & frequency	130	0.87	0.08
		128	0	0.08
		123	0	0.08
		109	0.13	0.78
	ho		0.24	0.39
	he		0.23	0.39
BOVIRBP	No. individuals		108	13
	Allele* & frequency	144	1	0.96
		140	0	0.04
	ho		0	0.08
	he		0	0.07
INRA040	No. individuals		100	13
	Allele* & frequency	240	0	0.04
		238	0	0.08
		202	0.32	0.81
		190	0	0.04
		188	0.68	0.04
	ho		0.39	0.23
	he		0.44	0.38
Average over 3 loci±SE	No. individuals		100.33±4.33	13.00±0.00
	A		1.67±0.33	3.67±0.88
	Ho		0.21±0.11	0.23±0.09
	He		0.22±0.13	0.28±0.11

*Molecular sizes (bases) refer to allele name
ho and *Ho* : observed heterozygosity.
he and *He* : expected heterozygosity.

while the BOVIRBP locus showed monomorphism in the Hokkaido population and two alleles in the Chiba population (Table 2). Between Hokkaido and Chiba populations there were some common alleles : two alleles at OarFCB193, one allele at BOVIRBP and two alleles at INRA040.

Observed heterozygosities (*ho*) of OarFCB193, BOVIRBP and INRA040 were 0.24, 0 and 0.39, respectively, in the Hokkaido population, and 0.39, 0.08 and 0.23, respectively, in the Chiba population. The average observed heterozygosity (*Ho*) was 0.21±0.11 for the Hokkaido population and 0.23±0.09 for the Chiba population (Table 2). The microsatellite genotype frequency significantly agreed with the Hardy-Weinberg expectation in each locus in the population except at INRA040 in the Chiba population ($p=0.06$) (Table 3). The difference between Hokkaido and Chiba was statistically significant (*Fst* value$=0.072$, $p=0.03$ in permutation tests).

DISCUSSION

Microsatellite analysis has more advantage than allozyme analysis and multilocus DNA fingerprinting, because of higher polymorphism and easier genotyping from a small amount of DNA. Nozawa *et al*. (1985) analyzed 28 allozyme loci from 20 individuals in the Hokkaido sika deer population and reported only two polymorphic loci with low variability : the average heterozygosity was 0.0158. By contrast, our results of microsatellite analysis showed much higher values ($Ho=0.21±0.11$) than their allozyme data (Table 2).

Abernethy (1994) indicated that the sika deer population introduced to Scotland showed monomorphism at the BOVIRBP locus and two alleles for OarFCB193. In the present study, the BOVIRBP locus of the Hokkaido population was also monomorphic and showed a low value (0.08) of heterozygosity in the Chiba population. These data suggest the BOVIRBP locus is not so hypervariable in the sika deer. Pépin *et al*. (1995) reported that the number of alleles for INRA040 were nine in the goat ($n=60$) and 44 in the cattle ($n>36$). Our results revealed that the sika deer in Japanese islands have at least four alleles for OarFCB193, five alleles for INRA040 and two alleles for BOVIRBP. In the present study, two other loci (INRA003 and INRA023) could not be PCR-amplified with primers reported by Pépin *et al*. (1995).

The genotype frequency significantly well agreed with Hardy-Weinberg expectations at all loci in both populations except for the INRA040 locus in the Chiba population (Table 3). The observed heterozygosities of OarFCB193 and BOVIRBP were same level as the expected heterozygosity, while the observed heterozygosity of INRA040 was lower than the expected heterozygosity (Table 2). Some heterozygotes such as null/normal genotypes, however, might have been counted as homozygotes of normal alleles, because it is difficult to distinguish homozygotes of normal alleles from null/normal genotypes with a single band of PCR product. Our results show that null allele may exist at INRA040 locus. Jarne and Lagoda (1996) suggested that null alleles may disturb population studies leading to underestimate of heterozygosity. The

INRA040 locus in the present study is likely in that case. From this reason, we compared heterozygosities of OarFCB193 and BOVIRBP between Hokkaido and Chiba. At both loci, heterozygosities of the Hokkaido population were less than those of the Chiba population (Table 2). Besides that, allele numbers at each locus in the Hokkaido population were much smaller than those of the Chiba population (Table 2). These results suggest a higher degree of homogeneity in the Hokkaido population. This supports a low genetic variety of mitochondrial DNA in the Hokkaido population, shown by our previous analysis (Nagata *et al*. 1998). Some bottlenecks of the sika deer recorded in Hokkaido history (Inukai 1952) could have induced such a low genetic diversity in the population.

The present results provide invaluable information for understanding genetic variety and history of the Hokkaido sika deer population, leading to the range of application of molecular genetics to conservation biology of the sika deer in Japan.

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The twinning rate of sika deer, *Cervus nippon,* on Mt. Goyo, northern Japan

Seiki TAKATSUKI

The University Museum, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan
Fax. +81-3-3815-7053, e-mail : taka@um.u-tokyo.ac.jp

Abstract. Knowing demographic parameters is important in order tounderstand the life history of mammals. As an example, the twinning rate of sika deer, *Cervus nippon*, on Mt. Goyo was determined based on 2,064 samples collected from 1981 to 1997. The sex ratio of single fetuses ($n=1,946$) was even (49.8% males and 50.2% females). Six pairs (0.29%) of twins were found. One pair was composed of male and female embryos, suggesting that at least some conceptions are dizygotic. It was concluded that twinning is rare in sika deer. This rate was similar to, or lower than, that found in red deer, *C. elaphus*.

Key words : *Cervus nippon,* Japan, reproduction, sika deer, twinning.

Many of the life history variables among mammals may be best explained on the basis of body size. The "Fast-slow continuum" theory (Eisenberg 1981, Stearns 1983, Martin and MacLarnon 1985), for example, has shown that smaller-bodied mammal species are not merely short-lived, but that they typically produce large litters of rapidly developing young, whereas larger mammals produce fewer young which develop slowly and live longer. There are, however, exceptions. Sika deer, *Cervus nippon*, for example, the males of which weigh 80 kg and the females of which weigh 50 kg, usually produce single offspring, while similarly sized *Odocoileus* species (Wallmo 1978) and the very much larger moose, *Alces alces*, the largest extant species of deer, regularly carry twins (Franzmann 1978). These differences may be better explained in terms of variation in species-specific habitat quality than in terms of mere body size. The habitats of *Odocoileus* species and moose are dominated by browse, which prevents detection by predators (Geist 1981).

In order to fully understand the life histories of mammals, a comprehensive range of parameters including body size, phylogenetic relations and habitat quality must be investigated (Wootton 1987, Harvey *et al*. 1989), and precise quantitative data is essential. Among the various life history variables, demographic information is one of the most important (Millar and Zammuto 1983, Fowler 1987).

Although the pregnancy rates and the age of weaning are fairly well known for sika deer (Koizumi 1992, Takatsuki 1992, Kaji 1995, Asada and Ochiai 1997), it was believed until recently that twinning did not occur in wild populations

(Feldhamer and Marcus 1994).   There have been, however, several reports of twinning both in captivity and in the wild.

   I have collected information on sika deer pregnancies since 1981 on Mt. Goyo, northern Japan, and have found several cases of twinning among more than 2,000 females.   The objectives of this paper, therefore, are to report on twinning in this population and to review previous reports on twinning in sika deer and in the closely related red deer, *C. elaphus*.

MATERIALS AND METHODS

   Sika deer were shot for pest control on Mt. Goyo in northern Honshu, Japan, between January and March each year from 1981 to 1997.   The deer carcasses were brought to checking stations where whole body weights were determined to the nearest 0.5 kg using spring scales prior to dissection.   As conception takes place during the autumn rut, fetuses were already well developed and generally weighed 100–900 g during the sampling period, thus it is believed that none were overlooked.   The rate of twinning was examined among 2,064 culled females.   The sex of the fetuses was determined by genital examination, though some fetuses ($n=124$) were too badly injured as a result of the shooting of their mothers for their sexes to be determined (Table 1).   The ages of the adults were determined by examination of the cementum annuli of the first incisors, or were estimated from the wear of the incisors (Ohtaishi 1976) according to known age-wear relationships (Takatsuki, unpublished).

RESULTS AND DISCUSSION

   Since sample sizes were small during the 1980s, they were rounded (Table 1).   Of the total of 2,058 single fetuses examined, 1,934 were sexed and among these the sex ratio was even (females 50.2%, males 49.8%, χ^2-test, $p > 0.05$).   If twins were added ($n=1,946$), the sex ratio was completely even (males and females=50.0%).

Table 1.   Number of pregnant females and sex of fetuses of sika deer on Mt. Goyo through 1981–1997.   f: female, m: male.

Year	Sex unknown	Single female	Single male	Twin f-f	Twin f-m	Twin m-m	Total
1981–89	5	93	107	0	1	1	207
1990	0	38	52	0	0	0	90
1991	1	89	70	0	0	0	160
1992	0	87	96	0	0	1	184
1993	7	197	123	0	0	0	327
1994	3	176	191	0	0	1	371
1995	28	102	102	0	0	1	233
1996	7	90	116	1	0	0	214
1997	73	98	107	0	0	0	278
total	124	970	964	1	1	4	2,064

Table 2. Information of females carrying twins.
*Figures in parentheses are estimated age from wear.

No.	Locality	Date of sampling	Body weight kg	Age year	Wear class
83068	Ofunato	Mar. 21, 1983	—	1.5	II
87012	Ofunato	Feb. 21, 1987	49.5	9.5	III$_3$
92229	Kamaishi	Feb. 29, 1992	52	(3.5*)	III$_1$
94553	Sanriku	Jan. 8, 1994	45	(10.5)	V
95186	Kamaishi	Feb. 26, 1995	50	(?)	?
96220	Takada	Feb. 1, 1996	45	(1.5)	I

Among the 2,064 pregnant females examined, six (0.29%) were carrying twins (Table 1), indicating that while twinning does occur, it is exceptional in this population.

Records of twinning are very rare among wild sika deer. Suzuki (1995) reported one example (1.1%) among 89 pregnant females in one Hokkaido population, and Uno (personal communication) found two sets of twins (3.4%) among 58 pregnant females in another, though he considered that this rate might be high because of his small sample size. Feldhamer and Marcus (1994) reported that a set of healthy sika deer twins was carried by one female among 54 females introduced to Maryland, USA. Five sets of twins (4.6%) among 108 births (Zuckerman 1953) and one set (1.20%) among 83 births (Haensel 1980) have been reported from German zoos. The sample size of the present study (2,064 females) was very much greater than in any of these cases thus the results from this study may be more reliable.

Among both Eurasian red deer and North American wapiti (elk) populations, both close relatives (both *Cervus elaphus*) of sika deer, twinning is also very rare (see review in Mitchell *et al.* 1977 and Sadleir 1987). Guiness and Fletcher (1971) recorded only one example among Scottish red deer, while other studies have indicated that twin embryos among red deer occur at rates ranging from less than 0.2% to 2.0% (less than 0.2%, Mitchell 1973 ; 0.2%, $n=1,690$, Kittams 1953 ; 0.2%, $n=1,186$, Flook 1970 ; 0.6%, $n=1,106$, Greer 1968 ; 1.2%, $n=875$, Korning and Vorreyer 1957), and 2.0% ($n=97$, Brna 1969).

During the present study, the combinations of twins were : one female-female set, one female-male set, and four male-male sets (see Table 1). Male and female twins were also reported among Hokkaido sika deer by Suzuki (1993), further indicating that at least some conceptions are dizygotic.

The data collected during the present study of the Mt. Goyo population provides no evidence for any particular tendency towards twinning in any particular locality, period, body weight, or age (Table 2). Since pregnancy among red deer is known to be affected by nutritional conditions (Mitchell *et al.* 1977), further studies of other populations are required to clarify what factors affect twinning in sika deer.

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The "Trace Recorder", a new device for surveying mammal home ranges, and its application to raccoon dog research

Yayoi Kaneko[1], Takeyoshi Suzuki[*2], Naoki Maruyama, Oichi Atoda[*], Nobuo Kanzaki and Masaki Tomisawa[*3]

Wildlife Conservation, Department of Ecoregion Science, Faculty of Agriculture, Tokyo Noko University, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-0054, Japan
* *Graduate School of Bio-Applications and Systems Engineering, Tokyo Noko University, 2-24-16 Nakamachi, Koganei, Tokyo 184-0012, Japan*
[1] Present address : *Landscape and Ecology Division, Environment Department, Public Works Research Institute, Ministry of Construction, 1 Asahi, Tsukuba City, Ibaraki 305-0804, Japan*
[2] Present address : *Yokogawa Electric Corporation, 2-9-32, Nakamachi, Musashino, Tokyo 180-0006, Japan*
[3] Present address : *Department of Information Engineering, Maebashi Institute of Technology, 460-1 Kamisatori, Maebashi, Gunma 371-0816, Japan*

Abstract. A new telemetric system known as the "Trace Recorder" was developed in order to reduce labor costs and to avoid radio-tracking location errors. It was first tested during studies of habitat utilization by raccoon dog, *Nyctereutes procyonoides viverrinus*, in Japan. The Trace Recorder (TR) consists of four separate units : beacons, recording units (RU), an automatic collar release system (ACRS) and a personal computer for processing data. The beacons emit 8 kHz magnetic signals periodically. A total of 600 different magnetic codes can be used in order to identify locations. The collar-based RU intercepts and records signals when the study animal is within 3 m of a beacon. The ACRS installed on the collar alongside the RU allows the collar to be released by a special code and recovered so as to facilitate the retrieval of stored data. In order of evaluate the capabilities of the trace recorder system, we used the TR in the analysis of the habitat use of a raccoon dog for 25 days between 16 November and 10 December 1996 in Hinode Town, suburb of Tokyo. Twenty-four beacons were set at along paths, at a garbage site, and at badger setts and animal latrines. The RU recorded 91 time units and durations of visits to trails and to some cores sites were collected. The TR system is capable of recording census data 24 hours every day for three months. The new TR system proved to be more accurate than current radio-telemetry equipment for recording frequency, duration and times of visits to target sites by the study animal.

Key words : automatic collar release system, home range, raccoon dog, telemetry system, trace recorder .

The wireless radio-telemetry system currently used in the study of free-ranging wildlife was first developed in the 1960s (Amlaner 1991). It has been widely used in relocating individuals and in measuring physical conditions of wildlife species (Mech 1983, Kenward 1987). Radio telemetry quickly proved a popular technique for tracking and studying small nocturnal, forest-living carnivores in Japan (Nakazono 1989, Ito 1992, Sasaki 1994, Tatara 1994, Yamamoto 1995). Locating animals is, however, usually quite laborious because of rugged terrain (Mech 1983, Ikeda 1985). In order to save on labor costs for relocating study animals, an automatic tracking system was devised (Doi 1985, Yoneda *et al*. 1988), however its use has been restricted by topographic condition and by access to electric power. Continuous location recording over-long periods of time is impractical by this method, especially of active, wide ranging species. Further, a triangular location technique of this current system could not avoid big errors because of users and terrain (Mech 1983).

Because of the high labor costs incurred while radio-tracking free-ranging animals, other methods have long been required by field researchers. In response to this need, the authors devised a new telemetric system known as the Trace Recorder or TR. The concept and the details of the circuitry involved in the TR have been submitted elsewhere (Suzuki *et al*. in press), hence in this paper, we introduce the systems practical capabilities and describe its application in a field study of the home range of a medium-size mammal, the raccoon dog, *Nyctereutes procyonoides viverrinus*.

THE TRACE RECORDER SYSTEM

Devised in 1996, the TR system consists of beacons, recording units (RU), automatic collar release system (ACRS) and a personal computer (Fig. 1). The fundamental unit of this system is composed of beacons and the RUs. Each beacon, set within a study area, emits 8 kHz magnetic field modulated by 14-bit unique serial codes and covers a range of 3 m radius. When six UM-1 batteries are connected in series, a beacon can emit signals for up to six months. At present, 600 beacon codes are distinguishable.

Collars weighing 130 g, including an RU are attached around the necks of animals. The RU is composed of a circuit board and two CR123A lithium batteries, which are connected in series and give the RU a working life of at least three months. Each collar's RU is capable of receiving 0.25 sec long magnetic signals from a beacon at 15 sec intervals, the RU records each signal along with the time in the unit's static random-access memory (SRAM). The SRAM provides a capacity of 16,000 time units. A time unit is defined as the duration of an RU's recording of the same signals emitted by a certain beacon ; this is the elapsed time between an RU-fitted animal arrival within the range of a certain beacon, and the time when it leaves the beacon's range. RU data recorded in the format of the following example "120 970915072015–970915185045" indicate that a study animal remained at beacon number 120 on 15th September 1997 from 07 : 20 : 15 (hours : minutes : seconds) to 18 : 50 : 45.
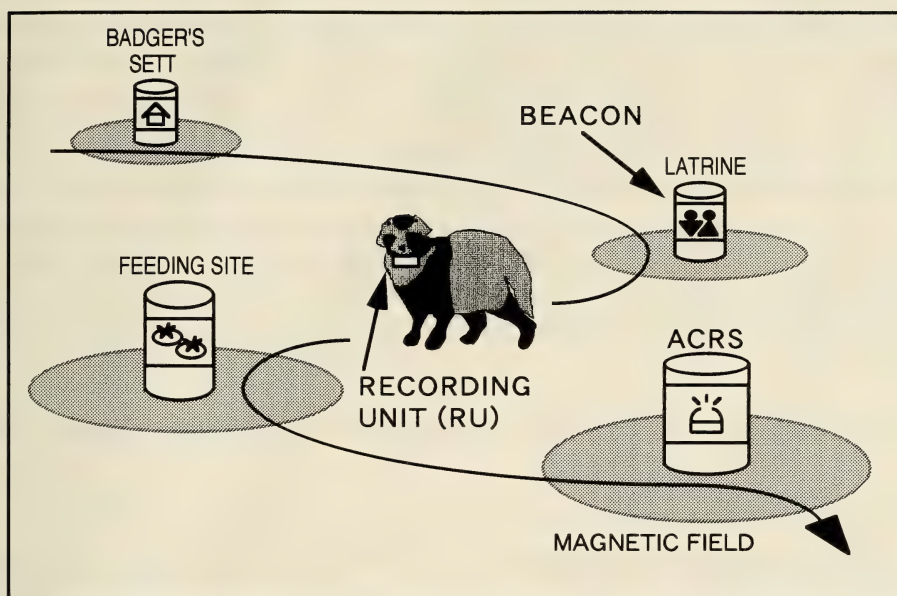
Fig. 1. Schematic outlining of the Trace Recorder system. When an animal equipped with a Recording Unit (RU) enters a beacon's 6 m in diameter magnetic field, the RU records its time and location. These are recorded using the beacon's unique ID number. At the operator's discretion, a special code will be sent via a beacon and the RU to the Automatic Collar Release System (ACRS) thereby releasing the collar immediately for recovery. By using a computer system, the accumulated information is downloaded from the RU.

The time unit are accurate to within 15 sec. After recovery of the RU, the memory is transferred to a personal computer and converted using the C language software for MS-DOS. The real time records of an animal's stay within the range of each beacon can then be reconstructed.

The ACRS is installed on collars along with RUs in order to be able to recover the stored data. When the RU receives a special code from a beacon, it transfers a special signal to the ACRS which then releases the collar immediately. The ACRS also triggers automatically releasing the collar when battery voltage falls so as to reduce any stress involved in carrying a collar to a minimum.

1. Animals weight

Collars with RUs attached weigh approximately 130 g, varying slightly depending on battery weight. If an acceptable upper limit to collar weight is 5% of the weight of a study animal (Kenward 1987), then this system is of use on any animal weighing more than 2.6 kg.

2. Applicability in home range research

When this system is applied to record home ranges, it is recommended to

use a method to the repeated-capture-in-traps (Jewell 1966), rather than the usual radio-tracking method.   That is, many beacons should be deployed in order to cover the home range of the animal to be examined.   The number of beacons required accuracy of the study.   The larger accuracy required, the more beacons are necessary.   Using this method, the primary issue is establishing where best to place beacons so that an area is thoroughly covered.   If plenty of beacons are available, this problem is easily resolved, however using many beacons is costly more than traditional radio-telemetry.   At the beginning of a research project, a home range should be roughly mapped based on detection using may be set out at core sites within the presumed home range such as at feeding sites, setts, latrines and animal paths in order to track the study animal(s) in detail.

APPLICATION TO HOME RANGE UTILIZATION OF A RACCOON DOG

STUDY AREA

The study area was located in Hinode Town, about 50 km west of the center of Metropolitan Tokyo.   Situated within the Pacific Ocean climate zone, the area has a mean annual temperature of 13.2°C, and a mean precipitation of 1,500 mm most of which falls in summer.   The area has a gentle topography of low hills covered with plantations of Japanese cedar, *Cryptomeria japonica* and Japanese cypress, *Camaecyparis obtusa*.   In the shallow valley bottoms, there are residential areas and cropland.   Other similar-sized mammals occurring in the study area include the badger, *Meles meles anakuma*, red fox, *Vulpes vulpes japonica*, and masked palm civets, *Paguma larvata*.

Some difficulties are experienced when using current radio-telemetry technique to track animals in such areas because of the reflection of radio waves by the mountainous topography and because of interference from amateur ham-radio communication systems.

METHODS

A young female raccoon dog, weighed 3.5 kg, was captured with a box trap and immobilized by ketamin hydrochloride.   A collar with both an RU and a radio-transmitter was attached to her and she was released on 16 November 1996.   From two to six radio-fixes were obtained each day using standard radio-telemetry techniques.   By 28 November, a total of 18 radio-fixes had been obtained.   Using the radio-fixes and the convex polygon method, the animal's home range was estimated to be of about 5.9 ha.   On 28 November 24 beacons were installed in and around this estimated home range at a feeding site (a garbage site) at badger setts (badger setts and resting sites of may be sometimes used by raccoon dogs), raccoon dog latrines and along animal paths (Fig. 2).

The authors interviewed local residents about frequency of waste disposal

at the garbage site in order to assess its potential significance to raccoon dogs. Since the tracking period by current radio-telemetry techniques was short and because the study animal was first caught outside her estimated home range, it was presumed that she might occasionally reappear outside the estimated range. Therefore, beacons were set at several possible sites outside the mapped range and she was also tracked using radio-telemetry techniques. The animal was recaptured on 23 December 1996, and data were downloaded onto a personal computer for analysis.

The daily activity period was defined as the period between the first and last recording beacon signals recorded each night. The non-active period was defined as the resting period. U and t-tests were used to compare the various activity patterns of this individual.

RESULTS AND DISCUSSION

1. Trace recorder data

The RU attached to this female raccoon dog recorded 91 time units from nine beacons over 12 days between 28 November and 10 December 1996. Six
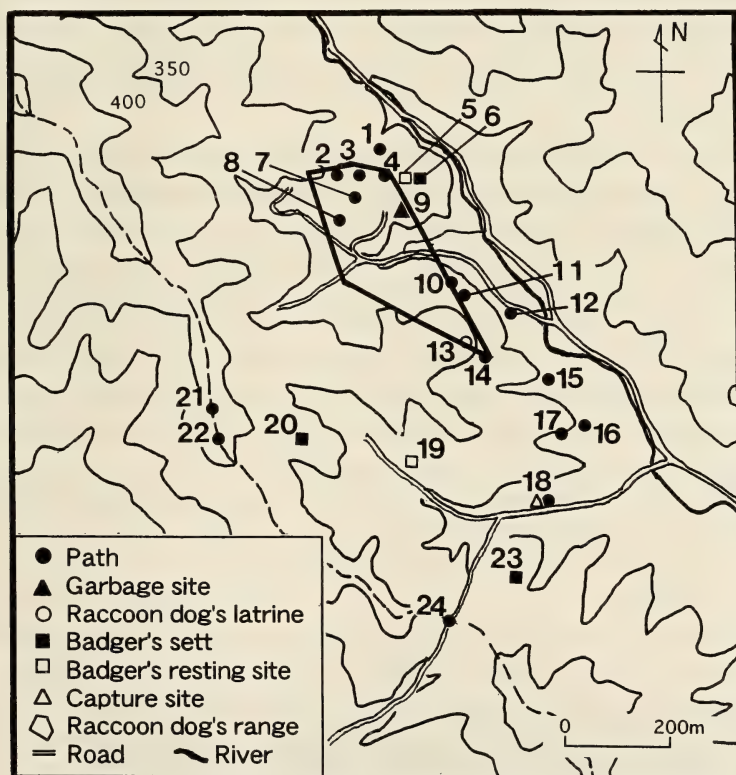
Fig. 2. The location of 24 beacons (with their IDs) in the range of a raccoon dog as defined by radio-telemetry between 16 and 28 Nov. 1996 in Hinode Town, Tokyo. Figures represent beacon IDs.

Table 1.   Locations and lengths of stay of a raccoon dog fitted with a recording unit in Hinode Town, Tokyo, between November 28 and 10 December 1996.

Beacon site	Duration of stay (hr : min : sec)	Number of time unit	Beacon ID
Path	0 : 10 : 15	4	7
Path	0 : 04 : 00	3	2
Path	0 : 02 : 45	8	4
Path	0 : 00 : 30	3	1
Path	0 : 00 : 15	1	3
Path	0 : 00 : 15	1	8
Badger's sett	1 : 09 : 45	8	6
Raccoon dog's latrine	0 : 06 : 30	2	13
Garbage site	8 : 24 : 15	61	9
Total	9 : 58 : 30	91	

beacons which were not recorded by RU
badger's resting site : 5, 19, badger's sett : 20, 23, path : 10, 11, 12, 14, 15, 16, 17, 18, 21, 22, 24

of these beacons were located along the paths, and the others were at a garbage site, a badger's sett and a raccoon dog's latrine (Table 1).   Eight of the nine beacons registered by the RU were from within the home range polygon determined by 36 radio-fixes obtained during the same period, only one beacon outside the range polygon was registered (Fig. 3).   The new home range polygon obtained during the TR study slightly north of that obtained prior to the use of the TR (see Fig. 2).

The 91 time units recorded were converted to a total of nine hours, 58 min and 30 sec.   The earliest RU time was at 17 : 03 on a day when sunset was at 16 : 28, and the latest was 06 : 24 on a day when sunrise was at 06 : 31.   During the nights of 7/8 December 1996, for example, her RU recorded 11 time units from three differrent beacons (Fig. 4).   On that night the study animal first appeared walking a path at 17 : 09 on 7 December, she then appeared at a garbage site, which she visited eight times between 17 : 12 on 7 December and 05 : 14 on 8 December.   She stayed there 15 sec to 42 min.   She made no visits other beacons while visiting the garbage site.   Finally, she appeared a badger's sett between 05 : 35 and 05 : 45 on 8 December.

The TR system clearly provided a very accurate method for recording the presence or absence at a target site.

2. Activity pattern

This raccoon dog proved to be active at night from immediately after sunset and just before sunrise throughout the 12-day study period.   She was active for 43.3 ± 8.7 (*SD*)% (range : 33.6-73.2%, $n=12$) of a 24 hour period, and the rested for 56.4 ± 8.4 (*SD*)% (range : 46.9-72.9%, $n=12$).   The active period was significantly shorter than the rest period (*t*-test, $p<0.01$).

The duration of time spent at the garbage site was 6.1 ± 7.9 (*SD*) % (range : 0.1-23.7%, $n=12$) of the activity period, and the time spent at the badger's sett was 0.9 ± 1.2 (*SD*)% (range : 0.5-4.2, $n=12$).
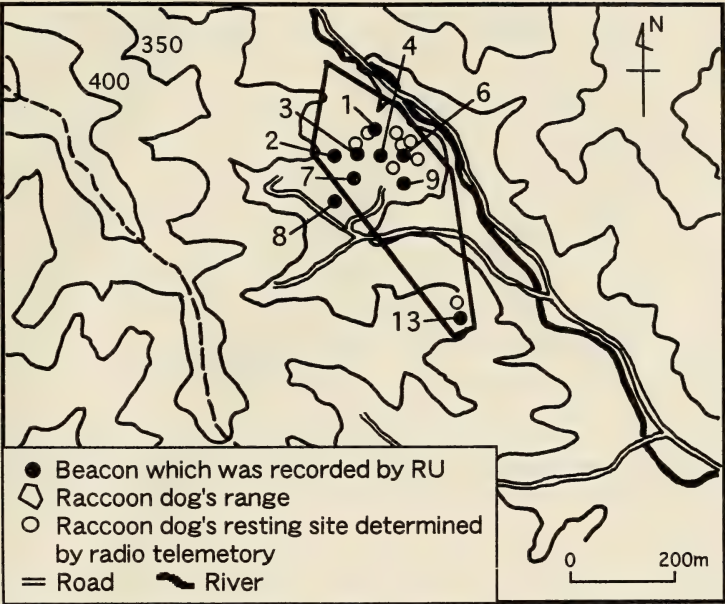
Fig. 3. The location of the nine Beacons recorded by a raccoon dog's RU and her home range drawn by 36 fixes with ten resting sites obtained by the radio-telemetry between 28 Nov. and 10 Dec. 1996 in Hinode Town, Tokyo. Figures represent beacon IDs.

Activity patterns varied at each of the sites where she was recorded (Fig. 5). Analyzing the data as a percentage of the total time spent at the badger's sett was divided into hourly intervals at the garbage site, the first peak in activity was from 18 : 00–19 : 00, and again 22 : 00 and 02 : 00. In the earlymorning, she made fewer visits to the garbage site. The percentage of time spent on paths reached a plateau between 21 : 00 and 03 : 00 with two troughs. The time spent at the badger's sett peaked between 05 : 00 and 07 : 00. Thus, it seems that this female raccoon dog first visited the garbage site, then walked through paths and visiting the garbage site again, and finally visited a badger's sett.

The TR system allows the collection of 24 hour-census data of target sites. Whereas when we used the radio-telemetry in Hinode Town, we had to watch and check the activity record on the recorder chart continuously because of noise from amateur ham-radio communication. Thus, the TR system saves considerable labor cost.
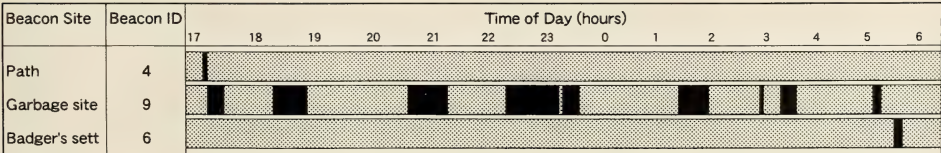
Fig. 4. An example of time units from the RU on the night of 7/8 Dec. 1996 in Hinode Town, Tokyo. Beacon ID 1, 2, 3, 7, 8 (path) and 13 (latrine) were not recorded.
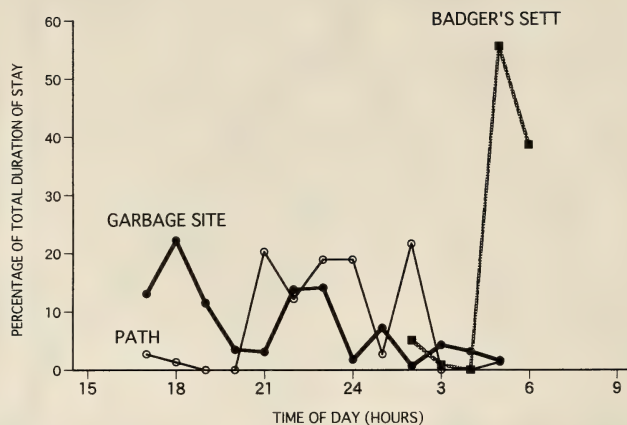
Fig. 5. Activity pattern of a female raccoon dog at target sites based on data from the Trace Recorder system in Hinode Town, Tokyo between 28 Nov. and 10 Dec. 1996.

3. Target site usage patterns

Visits to the garbage site were made intermittently, but every day between 28 November and 10 December 1996 (Fig. 6) with an average of 4.7±3.9 (*SD*) visits per day (range : 1-14, $n=12$), lasting on average 8.3±12.1 (*SD*) min per visit (range : 15-54 min, $n=61$). Three peaks were found at intervals of a few days during 12-day study period coinciding with when kitchen waste was disposed of at the site. The animal seemed to stay at the garbage site in order to search for food for significantly longer total periods on waste disposal days (112.9±47.5 (*SD*) min, $n=4$), than on non waste days (6.6±5.6 (*SD*) min, $n=8$, *U*-test, $p<0.01$). If she found no food, she left the garbage site after a short stay of 15 sec to just a few minutes.

At the end of her period of nocturnal activity, she visited the same badger's sett eight times on 8 of the 12 days (Fig. 7). She did not visit the sett on four mornings (3, 4, 5 and 9 December). Her visits to the badger's sett were usually short (8.5±4.3 (*SD*) min, range : 3.0-16.5 min, $n=8$). She was presumed to be looking for an opportunity to use the badger's sett as a resting site, but was unable to do because the sett was in use year-round by an adult female badger (Kaneko unpubl.).

For recording frequency, duration and time of the visit of an animal to target sites and places within a core area, the new TR system is more accurate than the current radio-telemetry systems. In particular, it is very useful in the study of short-term activity. Using the TR system in this study revealed that a female raccoon dog frequently checked the resources available to her such as a garbage site and a badger's sett. In the study area, an interval of radio-fixing may be around 15 min may be shortest, which is impossible for the current radio-telemetry to obtain the same accuracy. Furthermore, the new TR system greatly reduces the number of participants required to obtain data.
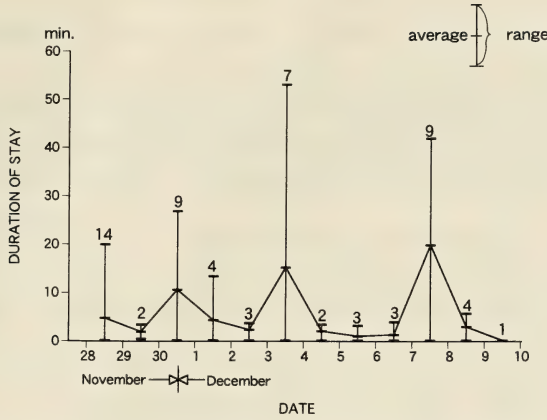
Fig. 6. The length of visits to the garbage site each night between 17:00 to 05:00 by a raccoon dog in Hinode Town, Tokyo between 28 Nov. and 10 Dec. 1996. Each figure on the vertical bar indicates the number of visits.

4. Detection of the opportunistic resting site

Radio-telemetry revealed that the study animal used ten resting sites in the rough proximity of badger's sett (Fig. 3). A careful search of the areas indicated by radio-telemetry, however, revealed no dens, and suggested that the study animal slept directly on the ground with or without cover. Eight of the ten resting sites were situated among bushes, one was located under a huge rock, and one was on a footpath on the shoulder of a mountain.

In conclusion, in this preliminary trial of the applicability of the TR system to wild animal studies, neither the number nor the density of beacons were taken into consideration. For the future development of this system and the methodology of its use, a spatial approach will be taken. It will be best to
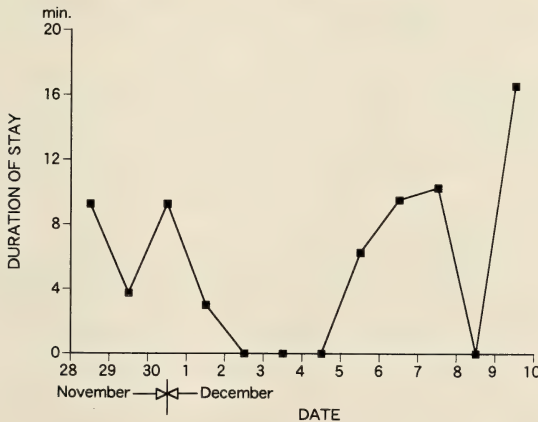
Fig. 7. Visits to a badger's sett by female raccoon dog in Hinode Town, Tokyo between 28 Nov. and 10 Dec. 1996. (Only one visit was made per day.)

devise a grid system that will effectively cover the suspected home range of the study animals.   The working period of the RU needs to be decided as does the best distance between the points of the grid in relation to animal home range size.   In addition to its advantages over current radio-telemetry techniques, a further advantage of the TR system over the grid trapping system is that it is less stressful to the study animals because they need only be trapped once.

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Short Communication

Measurements of the nasal sacs of individual common dolphin, *Delphinus delphis,* and Dall's porpoise, *Phocoenoides dalli*, by means of silicon reconstruction

Koji Nakamura[1], Tadasu K. Yamada[2] and Kenji Shimazaki[1]

[1] *Division of Marine Ecology, Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University, 1-1, Minatocho 3-chome, Hakodate, Hokkaido 041-8861, Japan*
Fax : +81-138-40-8860, e-mail : porpoise@pop. fish. hokudai. ac. jp
[2] *Division of Mammals and Birds, Department of Zoology, National Science Museum, 3-23-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan*

The toothed whales produce a wide range of species specific sounds with great differences between certain families. The acoustic characteristics of the echolocation sounds produced by the Phocoenidae and the Delphinidae are especially different (Kamminga *et al.* 1996). That of the delphinid common dolphin, *Delphinus delphis*, for example, is a broad-band, high-frequency sound of short duration ; the peak frequency range is 20–100 kHz, and the signal duration range is 50–150 μs (Evans 1973). In contrast, the echolocation signal of the phocoenid Dall's porpoise, *Phocoenoides dalli*, is a narrow-band, high-frequency sound of long duration ; the peak frequency range is 120–160 kHz, and the signal duration range is 180–400 μs (Awbrey *et al.* 1979, Hatakeyama and Soeda 1990).

The physical properties of the sounds produced by toothed whales are directly affected by the morphological characteristics of the air space in the head and by the sound-production mechanism (Aroyan *et al.* 1992). In order to understand how sounds are produced, and why there are such different acoustic characteristics between families, detailed information about the shape and volume of the air spaces is needed. In this paper, we describe a new experimental technique making it possible to obtain this information.

We used a silicon injection technique in order to determine the shape and dimensions of the air spaces in the nasal sacs of individual common dolphin and Dall's porpoise. Two specimens, one common dolphin (male, B. L.=157 cm, M30116, National Science Museum, Tokyo) and one Dall's porpoise (sex and B. L. unknown, collected at Otsuchi, Japan) were examined. The heads of both specimens were frozen before examination. Prior to injecting silicon, the larynx and the surrounding muscle complex of each animal was removed and the head was turned upside down. We then poured 200 ml of KE12 silicon (Shin-etsukagaku Kogyo Co., Tokyo, Japan) into the bony nares and kept the heads in position for eight hours. KE12 silicon is relatively tough, polymerises

at room temperature when mixed with one or more catalysts and solidifies after approximately eight hours at 25°C.

The silicon is prevented from entering the air space between the external nares and the blowhole by the nasal plug muscle. This muscle originates chiefly on the premaxilla anterior to the premaxillary sac with a few fibers arising in the connective tissue band along the margin of the premaxilla lateral to the sac (Lawrence and Schevill 1956).

Following solidification of the silicon, the heads were returned to their natural position with the blowhole pointing upwards in order to reconstruct the air space between the external nares and the blowhole. This was done by injecting 100 ml of silicon into the blowhole using a 200-ml plastic syringe with a surgical tube inserted 2–3 cm into the nasal passage. Air in the deep nasal sac was ejected by the pressure of the fluid silicon passing through the blowhole. This second injection of silicon was also allowed to harden for eight hours at room temperature. The hardened silicon was finally removed by dissecting the heads.

Examination of the silicon cast of the nasal sacs of the common dolphin specimen (see Fig. 1) revealed that the left vestibular sac measured 3.5 cm along the anterior-posterior axis, and 2.9 cm transversely, whereas the right vestibular sac measured 4.0 cm by 3.5 cm. The anterior nasofrontal sac was 4.0 cm long, and the posterior nasofrontal sac 3.8 cm long. Eight small diverticula were found between the anterior and posterior nasofrontal sacs. The right accessory sac was 2.2 cm long. The premaxillary sacs were measured 6.3 cm by 2.5 cm (left), and 8.0 cm by 4.7 cm (right). Silicon was not injected into the left nasofrontal sac. The total volume of the nasal air space of this individual common dolphin was found to be 33.3 cm³.

Examination of the silicon cast of the nasal sacs of the Dall's porpoise specimen (see Fig. 2) revealed that the left vestibular sac measured 4.5 cm along the anterior-posterior axis and 4.2 cm transversely, and that the right vestibular sac measured 7.5 cm by 5.2 cm. The left premaxillary sac measured 4.5 cm along the anterior-posterior axis and 2.4 cm transversely, whereas the right premaxillary sac measured 3.5 cm by 2.3 cm. Silicon was not injected into the nasofrontal sac or the posterior nasal sac. The volume of the nasal air space of this individual Dall's porpoise was found to be 61.5 cm³. The silicon injection technique proved an effective way of examining the nasal air spaces in two different species of odontocetes, and revealed that the Dall's porpoise has almost twice the volume of nasal air space, and larger vestibular sacs than the common dolphin.

The results from two-dimensional computer modelling suggest that the source of echolocation signals may be the dorsal burse below the vestibular sacs (Aroyan *et al.* 1992). Furthermore, in order to understand why different toothed whale families produce sounds with different physical characteristics, detailed measurements of the air spaces in their heads are needed. Reconstruction of the air spaces in the heads of odontocetes using silicon facilitates the detailed measurement of both the shape and volume of spaces such as the small
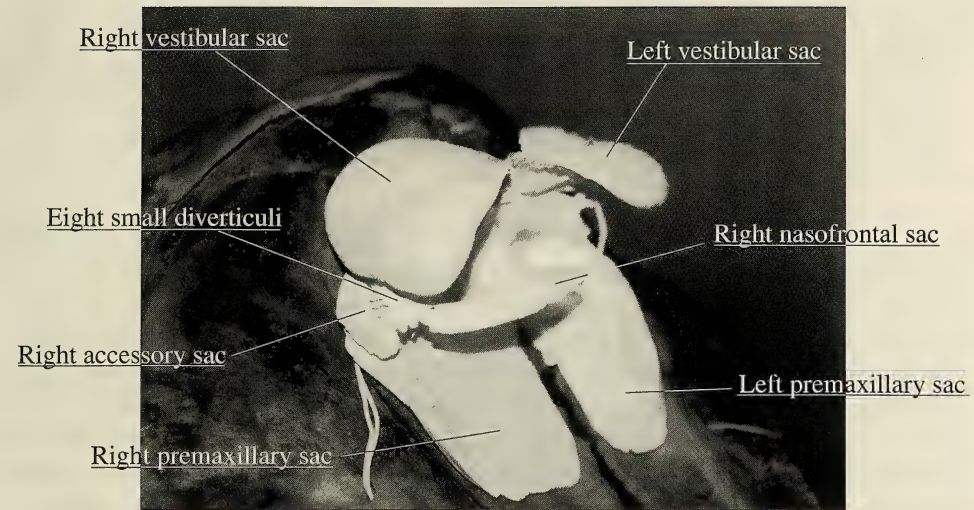
Fig. 1. Silicon reconstruction on the nasal sacs on a common dolphin skull. Skull width (Zygomatic width)=16.9 cm. Skull length (Condylobasal length)=41.3 cm.
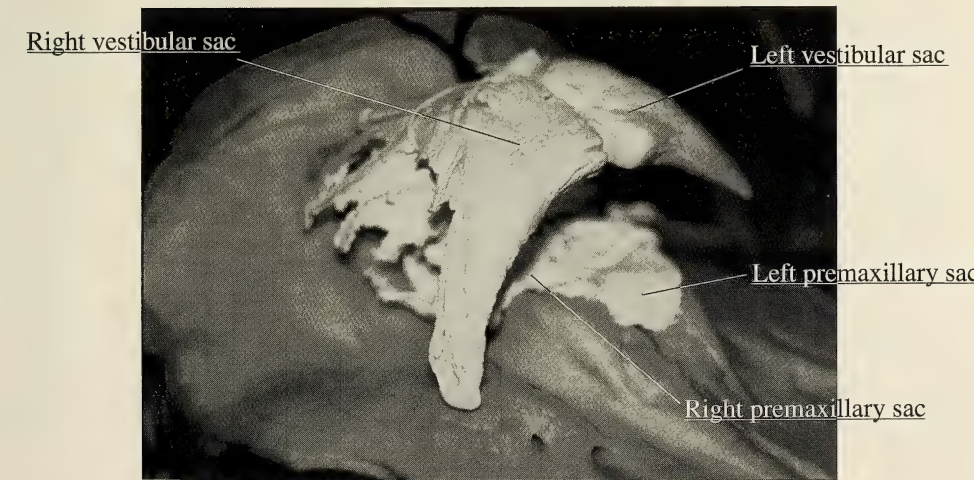
Fig. 2. Silicon reconstruction on the nasal sacs on a Dall's porpoise skull. Skull width (Zygomatic width)=18.6 cm. Skull length (Condylobasal length)=33.5 cm.

nasofrontal diverticula, and this technique may prove valuable in studies of sound production. In recent years, new medical imaging techniques such as x-ray computed tomography (CT) and magnetic resonance imaging (MRI) have been used to describe the internal details of the foreheads of toothed whales (Cranford 1988, Amundin and Cranford 1990, Amundin 1991, Cranford *et al.* 1996). Future research into the sound production mechanisms of odontocetes may benefit from incorporating both silicon reconstruction of nasal regions and CT and MRI medical imaging techniques along with three-dimensional computer modelling.

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Short Communication

Bark-stripping of tankan orange, *Citrus tankan*, by the roof rat, *Rattus rattus*, on Amami Oshima Island, southern Japan

Tatsuo YABE

Kanagawa Prefectural Public Health Laboratories, Asahi-ku, Yokohama, 241-0815, Japan
Fax. +81-45-363-1037

In 1997, roof rats, *Rattus rattus*, damaged the bark of cultivated tankan orange, *Citrus tankan* Hayata, trees over a wide area of the central part of Amami Oshima, an island in the Nansei Shoto archipelago of southern Japan. This was the first time that tankan farmers had experienced such damage in more than 30 years of cultivation of the fruit. At first, it was believed that the introduced mongoose, *Herpestes* sp., had damaged the trees, but later, from the appearance of the tooth marks on the trees, it was surmised that the rats were responsible. The damage which occurred from early April until early October 1997 was found in an area where other potential mammalian culprits such as *R. norvegicus, Tokudaia osimensis* and *Diplothrix legata* were known to be absent.

Bark-stripping by *R. rattus* has been reported elsewhere (*e. g.*, Maeda 1982, 1985, Santini 1987), but has not previously involved the tankan orange, making the damage caused on Amami Oshima Island notable. In this paper the bark-stripping activity of the roof rat is described, and the data on their movements around a tankan orchard, their food habits and their age composition are examined.

STUDY AREA AND MOTHODS

Amami Oshima Island is a 712 km² island situated at 28°10-30' N, 129°10-45' E (Fig. 1). It is situated in the sub-tropical zone, and has a warm, humid climate with mean monthly temperatures ranging from a low of 14.2°C in January to a high of 28.4°C in July and an annual mean temperature of 21.3°C. Rainfall amounts to 2,871 mm a year.

In mid-September 1997, I carried out a study in a tankan orchard where severe damage occurred (Fig. 1). The orchard situated in the Naze City administrative district had a total area of about 1.2 ha, which was divided into several plots by woods composed of evergreens such as *Castanopsis cuspidata, Symplocos* spp., *Melia azedarach* and *Pinus luchuensis*.

Rats were studied by trapping and tracking. They were captured in 29 live traps set for one night at 3-5 m intervals along the edge of a wood that faced an orchard plot of about 400 m². They were tracked using fluorescent
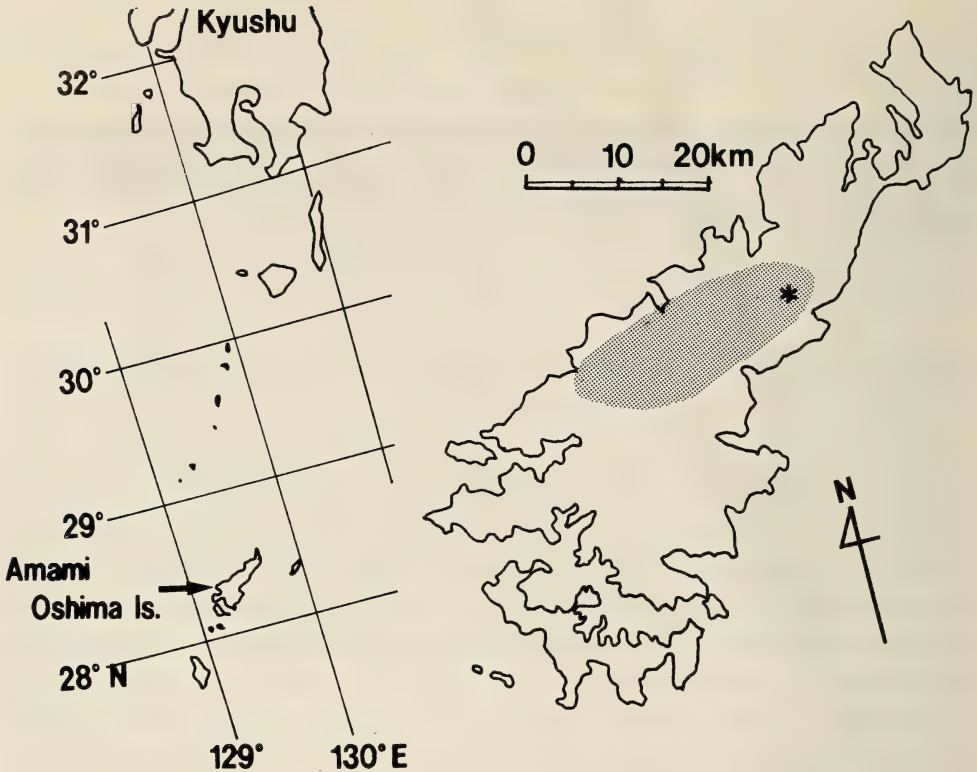
Fig. 1.   Amami Oshima Island, showing the study site (asterisk) and the approximate area including Naze City, Yamato Village and Sumiyo Village, where bark-stripping by rats occurred (shaded).

pigments following the method described by Lemen and Freeman (1985). Trapped rats were put into bags containing fluorescent pigments, gently shaken and released in the morning.   During the following night, from circa 23 : 00 onwards, their trails were detected with a 4W UV-lantern.

Ninety snap traps were also set for one night at 3–5 m intervals along the same woodland/orchard boundary near where the live traps had been set. Snap traps were baited with sweet potatoes covered with peanut butter and honey.   Specimens were dissected in the laboratory, and their stomachs were removed for closer examination under a stereoscopic microscope following the method by Yabe (1979).   The volume that different food items contributed to each stomach's contents (excluding bait) was estimated, and the mean volume of each food item was calculated for all stomachs examined.   Rats were aged on the basis of their eye-lens weights using Tanikawa's formula (Tanikawa 1993), and individuals three months of age or older were defined as adults.

RESULTS AND DISCUSSION

Bark-stripping by rats has previously been reported from both Europe and

Southeast Asia.   In Central Italy, the roof rat was considered to be responsible for heavy bark-stripping activity on *Pittosporum tobira* shrubs in urban parks (Santini 1987), although the reason for the activity remained uncertain.   Maeda (1982, 1985) reported bark-stripping of ipil-ipil, *Leucaena leucocephala,* trees by *R. rattus mindanensis*, although in my opinion the species was identified erroneously given that the specimens collected had white tipped tails and weighed 250 –400 g, characteristics typical of *R. everetti*, not *R. rattus*.

In Amami Oshima Island, I confirmed that the species de-barking tankan trees was the roof rat by finding their tooth marks on trees, their hairs in feces found below trees as well as by direct trapping.   The rats stripped bark mainly from near the bases of the trunks of young trees less than five years old and from the branches of older trees.   Most of the damaged trees were completely girdled (Fig. 2A).   The damage extended to all parts of the orchard surveyed, even to trees at the center, some 20 m from the nearest forest edge.

Tracking of two rats dusted with fluorescent pigments revealed that they had moved about 15–20 m through the woods along the edge of the tankan orchard before turning into the orchard and attacking the tankan trees about 5–8 m inside.   The tankan orchard apparently provided the rats with little shelters because there were no ground cover, whereas the surrounding woods probably provided shelter, preferred runways, as well as foods such as acorns.

A total of 21 rats (18 females and 3 males) were collected over 90 trap-nights around the tankan orchard.   Seventeen of the 18 females were adult, but
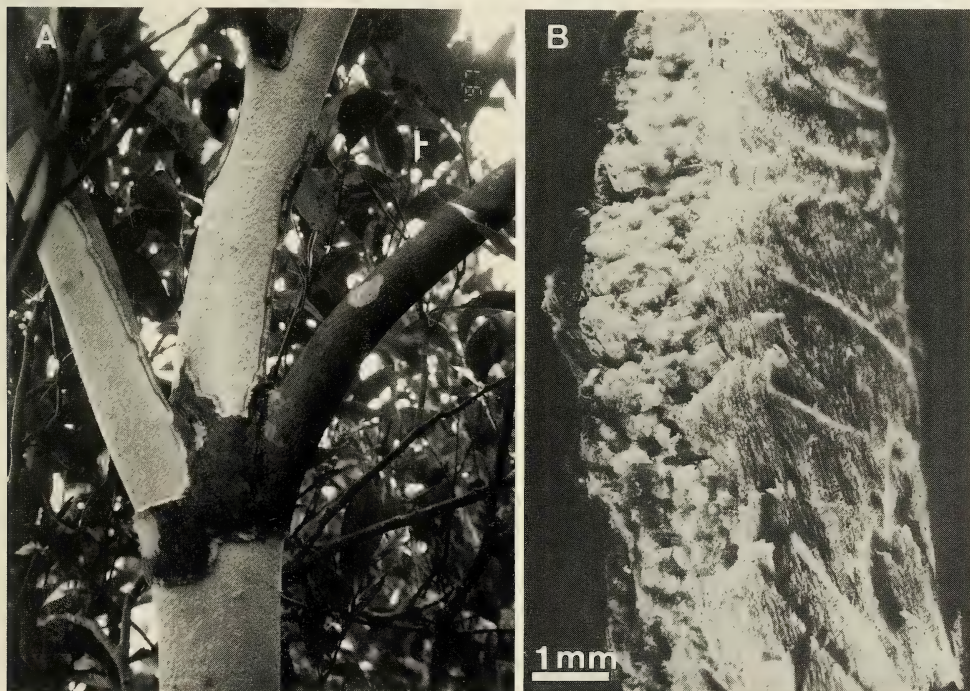
Fig. 2.   Bark-stripping of tankan orange, *Citrus tankan*, trees (A), and tooth marks on the inside of fallen bark chips (B).

Table 1.   Age composition of roof rats trapped around a tankan orchard.

Age in month	No. of individuals		
	Males	Females	Total
2	0	1	1
3	0	0	0
4	0	2	2
5	0	1	1
6	1	0	1
7	0	4	4
8	1	3	4
9	0	3	3
10	0	0	0
11	0	1	1
>12	1	3	4
Total	3	18	21

none were pregnant.   The majority of individuals (52%, 11 of 21) were 7–9 months old (see Table 1) indicating that a major breeding season had lasted from December 1996 to February 1997.   S. Hattori (pers. comm.) was of the opinion that the roof rat population had exploded during the previous winter owing to a heavy crop of acorns.

Tankan phloem, which was identified by the characteristic sieve areas of the tissue, was found in two (11%) out of 18 stomachs examined, however, no trace of outer bark was found in those stomachs.   Tooth marks left on the inside of the bark chips clearly indicated that rats chewed the phloem contained in the bark chips as well as on the tree surface (Fig. 2B).   The fact that rat feces were filled with phloem fibers indicated that they digested phloem incompletely, and presumably absorbed only the sap.   Seeds and fruits accounted for 30.1% of the stomach contents in volume, and phloem accounted for 8.9% (Fig. 3).
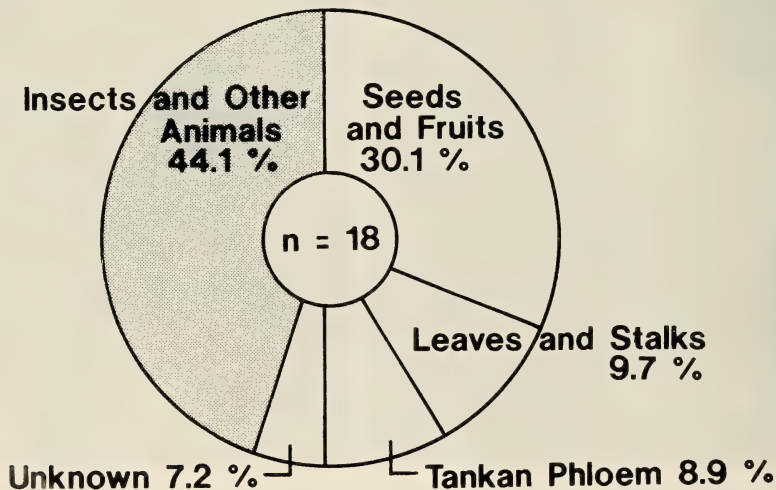
Fig. 3.   The stomach contents of roof rats in Amami Oshima Island.

The preferred diet of the roof rat has been shown to consist of seeds and fruits in general (Yabe 1979), although it will switch to more succulent foods such as herb stems in order to obtain moisture (Yabe 1982). Stomach analysis of specimens trapped during this study confirmed that seeds and fruits were primary food source of the roof rats, and showed that phloem was at most a supplementary, not a substitute food source. I conclude that roof rats stripped the bark of the tankan orange trees to obtain the sap in the phloem. The reason for this activity remains uncertain, though they may have involved accessing extra moisture and/or extra nutrients.

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Short Communication

The structure of the pawpad lamellae of four *Rattus* species

Tatsuo YABE[1], Puangtong BOONSONG[2] and Sermsakdi HONGNARK[2]

[1] *Kanagawa Prefectural Public Health Laboratories, Asahi-ku, Yokohama 241-0815, Japan*
Fax. +81-45-363-1037
[2] *Entomology and Zoology Division, Department of Agriculture, P.O. Box 9-34, Bangkok 10900, Thailand*

The structure and function of the pawpad lamellae of *Rattus* species relate directly to their behavior (Brooks and Rowe 1987). Thus the pawpads of climbing species such as the roof rat, *R. rattus*, have evolved numerous lamellae to provide better gripping and clinging power, whereas digging species such as the Norway rat, *R. norvegicus*, have smooth pawpads. Pawpads have only been described previously, however, as either finely lamellated or nearly smooth (Musser 1973, Marshall 1977, Corbet and Hill 1992), and no detailed studies of the structure of the lamellae have been carried out. In this paper, we describe the histological features of the lamellae and relate them to the differing behaviors of two climbing *Rattus* species (*R. rattus* and the Polynesian rat, *R. exulans*) and two digging species (the ricefield rat, *R. argentiventer* and *R. norvegicus*).

MATERIALS AND METHODS

Specimens of *R. rattus* and *R. norvegicus* from Japan, and of *R. exulans* and *R. argentiventer* from Indonesia and Thailand were used in this study. The majority of these specimens were laboratory reared in Miyazaki Medical College and Ikari Corporation with the remainder killed just after capture in fields or buildings.

The largest pawpads were those of the outer metatarsals so these were removed for lamellar analysis. Pawpads were surgically excised, fixed in 10% formalin, washed in tap water and dehydrated in a graded series of ethanol. Specimens were then immersed in isoamyl acetate and dried with liquid CO_2 in a critical point dryer. They were mounted on a scanning peg using a piece of conductive tape coated to 30 nm with gold-palladium in a DC sputtering apparatus, and observed at 10 kV in a JSM 5400LV scanning electron microscope (SEM). Microscopic photographs were taken at a magnification of 100.

Histological preparations were made from pads fixed in 10% formalin. The fixed pads were removed from the hind feet and embedded in paraffin using standard histological procedures. The pads were cut into serial sections

vertical to the lamellae at 8-10 μm intervals and stained with hematoxylin-eosin.   One serial section from the middle part of the pad was selected for detailed examination and measurements.   The height and width of the lamellae were measured with an ocular micrometer.   The image was then projected onto a screen and the angle of the lamellae was measured with a protractor. The maximum height of the stratum corneum was defined as Ch, the maximum width of lamellae as Lw, and the average angle of 10 pits on corneous, lucid or granular layers as θ in radians (Fig. 1).   Because the pits on the corneous layer were often split, those on the lucid or granular layers were more suitable for measuring angles from.   The lamellae on the front of the pawpads were excluded from these measurements because they often had irregular pit angles, width and height.   Values of Ch, Lw and θ from 10 specimens were averaged for each species.   Statistical analyses of these values were made by using the Kruskal-Wallis analysis of variance of ranks followed by the Tukey test.

RESULTS AND DISCUSSION

Among mammals, the pattern of the peculiar outer surface of the corneous layer is generally affected by the lower epidermal layers and the dermis (Sokolov 1982).   The four species of *Rattus* also have lamellae consisting of a superior corneous layer (stratum corneum) parallel to the underlying lucid (stratum lucidum) and the granular (stratum granulosum) layers (Fig. 1).   Pit angles from the lucid or granular layers could therefore be substituted for those from the corneous layer.   Keratin plates of the corneous layer were found to be arranged in columns as was suggested by Sokolov (1982), and each lamella was distinguishable in the columns.

Both histological sections and SEM photographs showed that whereas *R. rattus* and *R. exulans* had extemely-developed lamellae, *R. argentiventer* had moderately-developed lamellae and *R. norvegicus* had only poorly-developed lamellae (Figs. 1 and 2, Table 1).   The Kruskal-Wallis analysis of the four species revealed significant differences among them in Ch ($d.f.=3$, corrected $H=11.0$, corrected $p<0.05$), Lw ($d.f.=3$, corrected $H=18.5$, corrected $p<0.01$), and θ ($d.f.=3$, corrected $H=31.6$, corrected $p<0.01$).   The Tukey tests showed that *R. rattus* had significantly greater Ch's than the three other species (Studentized range $Q=3.80$, $d.f.=36$, number of treatments $a=4$, $p=0.05$, significant difference $D=81.4$), while *R. norvegicus* had significantly greater Lw's than those of the other species ($Q=3.80$, $d.f.=36$, $a=4$, $p=0.05$, $D=30.5$). The mean pit angles (θ) were in the order : *R. rattus* = *R. exulans* > *R. argentiventer* > *R. norvegicus* ($Q=3.80$, $d.f.=36$, $a=4$, $p=0.05$, $D=0.32$), which confirmed the observations made with the SEM.

Musser (1973) had noted that the pawpads of digging *Rattus* species were flush, whereas those of good climbers such as *R. rattus* protruded from the sole. This study has confirmed that the prominent pawpads of *R. rattus* are due to the thick corneous layer.   It appears therefore that among *Rattus* spp. good climbers have prominent pawpads or a thick corneous layer as well as finely
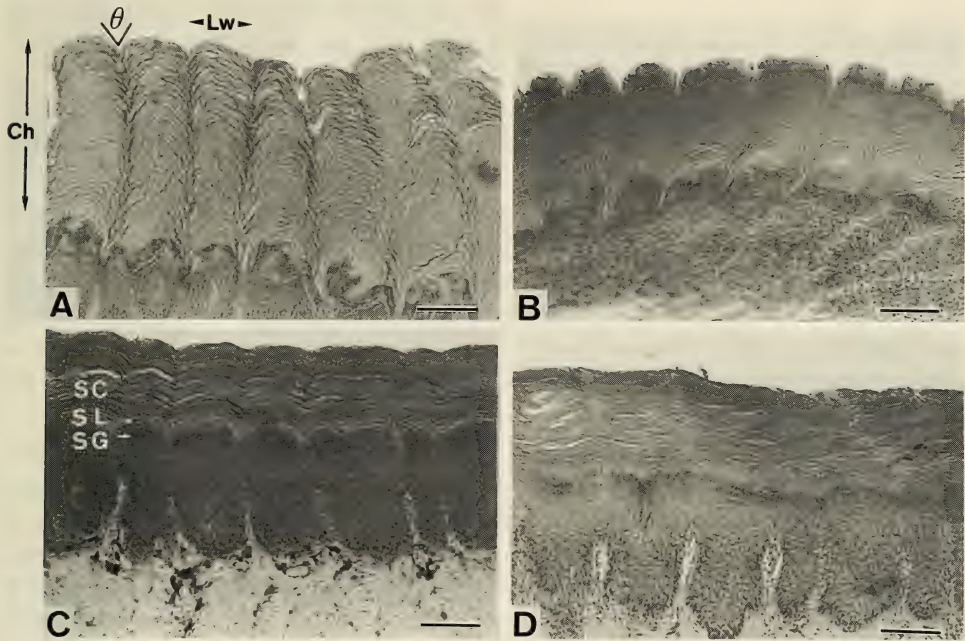
Fig. 1.   Structure of pawpad lamellae (×100) of (A) *Rattus rattus*, (B) *R. exulans*, (C) *R. argentiventer* and (D) *R. norvegicus*, showing measurements taken.   The scale indicates 100 µm.   SC : stratum corneum, SG : stratum granulosum, SL : stratum lucidum, Ch : height of corneous layer, Lw : width of lamella, θ : angle of pit on lucid or granular layers.
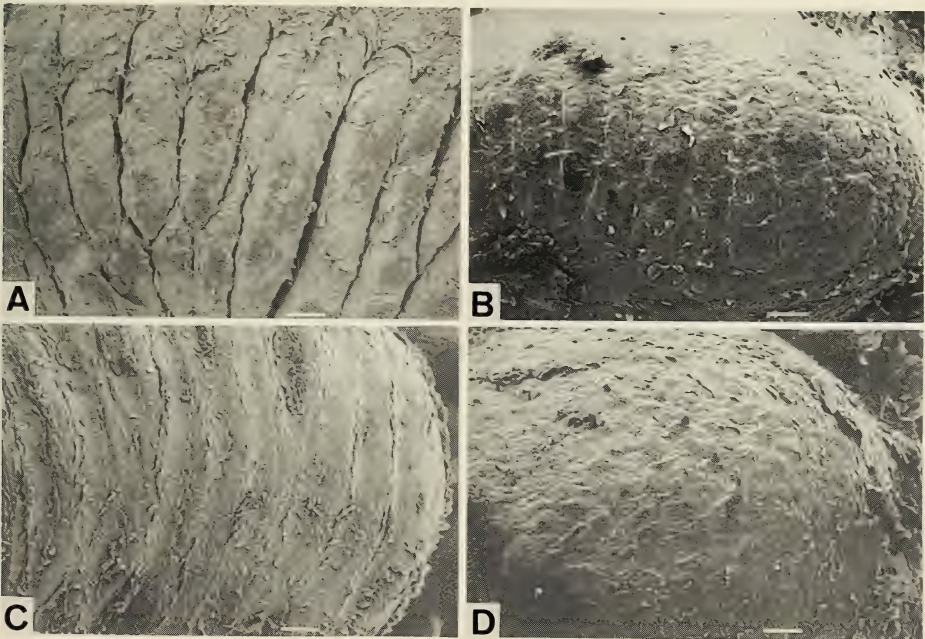
Fig. 2.   SEM photographs (×100) of pawpad lamellae of (A) *Rattus rattus*, (B) *R. exulans*, (C) *R. argentiventer* and (D) *R. norvegicus*.   The scale indicates 100 µm.

Table 1.   Maximum height of corneous layer (Ch), maximum width of lamellae (Lw), and average pit angle between lamellae (θ) of the pawpads of four *Rattus* species.

Species	n	Ch$\pm$SD (μm)	Lw$\pm$SD (μm)	$\theta\pm$SE (radian)
R. rattus	10	303$\pm$73[a]	137$\pm$21	1.06$\pm$0.17
R. exulans	10	200$\pm$67	123$\pm$21	1.08$\pm$0.16
R. argentiventer	10	203$\pm$45	153$\pm$20	1.93$\pm$0.35[a]
R. norvegicus	10	216$\pm$69	185$\pm$32[a]	2.66$\pm$0.07[b]

[a] Significantly larger than the others except "b" in the same column ; [b] significantly larger than "a" (Tukey test, $p=0.05$).

lamellated pawpads.   These finely lamellated pawpads have steep lamellar pits and narrow lamellae : the steeper the lamellar pits and the narrower the lamellae, the more grip they provide for clinging or climbing.

In conclusion, our examination of the histological features of *Rattus* pawpad lamellae has shown that they differ in structure corresponding with the behavior of the species.   The pawpads of digging species such as *R. norvegicus* are characterized by a thin corneous layer, shallow lamellar pits and broad lamellae.   In contrast, the pawpads of climbing species such as *R. rattus* are characterized by a thick corneous layer, steep lamellar pits and narrow lamellae.

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Editor's Acknowledgments

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Geist, V. 1982. Adaptive behavioral strategies. *In* (J. W. Thomas and D. E. Toweill, eds.) Elk of North America. pp. 219−277. Stackpole, Harrisburg.

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Mammal Study

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Seasonal changes in body weight of female Asiatic black bears under captivity

Yukihiko Hashimoto[1] and Aiko Yasutake[2]

[1]*Laboratory of Wildlife Biology, School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8654, Japan*
[2]*Wildlife Management Office, Kawasaki 214-0011, Japan*

Abstract. Though body weights of bears are known to change seasonally responding to nutritional conditions, there is little information on the body weight and nutritional condition of Asiatic black bears, *Ursus thibetanus*. We weighed seven female Japanese black bears, *U. thibetanus japonicus*, under captive condition from May to December, 1995. Although the body weights did not differ significantly between years, three seasonal phases were distinguishable according to the increase rate (r_w). During Phase I, mean body weight increased gradually from May (46.2 ± 4.4 kg, mean $\pm SD$, $n=6$) to August (57.3 ± 3.5 kg, $n=6$: $5\% < r_w < 10\%$). From August to November, the mean body weights were stable (59.4 ± 4.3 kg, $n=4$: $r_w < 5\%$). Contrary, body weights increased rapidly during November and December (68.4 ± 4.7 kg, $n=5$: $10\% < r_w$). The gradual body weight increase in the Phase I was probably because of sufficient food and lower energy expenditure, while the rapid increase in Phase III seems to be an adaptation for hibernation.

Key words: Asiatic black bear, body weight, hyperphagia, seasonal change, *Ursus thibetanus*.

Studies of the nutrition of wild animals are important, because nutrition relates significantly to wildlife ecology, physiology, conservation biology and many other fields of study (Robbins 1993). Studies on the nutrition of captive animals may sometimes provide valuable information, and are often used to obtain valuable base-line information on wildlife nutrition (Mautz 1978; Robbins 1993). Body weight is one of the simplest and clearest parameters responding to the changing nutritional condition of wild animals (Tsubota 1998).

The Asiatic black bear, *Ursus thibetanus*, is a medium-sized bear that is widely distributed in south-eastern and eastern Asia (Servheen 1990). In the temperate zone of Japan, the diet of the Japanese black bears, *U. thibetanus japonicus* varies seasonally in response to changes in food availability (Hashimoto and Takatsuki 1997), and it hibernates during winter. These ecological features are similar to those of the American black bear, *U. americanus*, with which it shares similar seasonal changes in its nutritional condition and physiology (Jonkel and Cowan 1971; Nelson et al. 1983; Hellgren et al. 1989; Hellgren et al.

[1]*E-mail: yukih@uf.a.u-tokyo.ac.jp*
[2]*Present address: 754-84, Simodzukise, Sanda 669-1413, Japan*

1993).

As there is only very limited information on the seasonal nutritional condition of wild or captive Asiatic black bear, the purpose of this study was to clarify the pattern of seasonal changes in the body weight of captive Asiatic black bear as a first step in the study of the nutrition of this species.

Materials and methods

This study was conducted at the Institute of Japanese Black Bear in Ani in Akita Prefecture Japan, during 1994 and 1995. There, captive Japanese black bears, were fed the same artificial food in approximately similar amounts every day from May to early October. Rations were increased, however, during October, November and December because the bears' appetite appeared to increase during this period, and because they occasionally fought seriously over food at this time of year. The precise amounts of food given, however, were not recorded. From the middle of December until April the bears hibernated without feeding.

Individual bears were weighed monthly as possible as we could. Bears were first isolated in an enclosure, tranquilized and immobilized using darts dosed with ketamine hydrochloride (11–13 mg/kg) and xylazine hydrochloride (1.1–1.3 mg/kg), then weighed to the nearest 0.5 kg using a spring balance.

In order to address whether bears grew between 1994 and 1995, the weights of five adult females (more than five years old in 1994) were compared between 1994 and 1995. One bear (Y45) was weighed in mid September, and four bears (W32–W35) were weighed in mid October 1994. The weights in 1994 were compared with those of the same month in 1995.

In 1995, seven adult females were studied, however data were not obtained every month because some bears did not enter the enclosure in some months (see Appendix 1). Body weights are presented as means$\pm SD$ (kg), and rates of body weight increase (%, r_w) were calculated using the equation:

$$r_w = \frac{W_i - W_{i-1}}{W_{i-1}} \times 100$$

$\qquad$ W_i: Mean body weight in month i.

Analysis of variance was used to compare monthly weights from May to December, and when appropriate, Tukey's multiple comparison test was applied.

Results

Although the body weights of the bears did not differ significantly between years (60.5$\pm$5.0 kg in 1994, 60.3$\pm$3.1 kg in 1995, Student's t test, $t=0.225$, $df=3$, $P=0.836$), they did change significantly during the course of a single year (Table 1, $F=15.190$, $df=7$, $P<0.001$). Body weights ranged from as low as 46.2$\pm$4.4 kg in May to as high as 68.4$\pm$4.7 kg in December. Mean body weight increased consistently month by month except from August to September when weight losses of 0.05 kg were recorded.

Three seasonal phases (I, II and III) in mean body weight were distinguishable in rela-
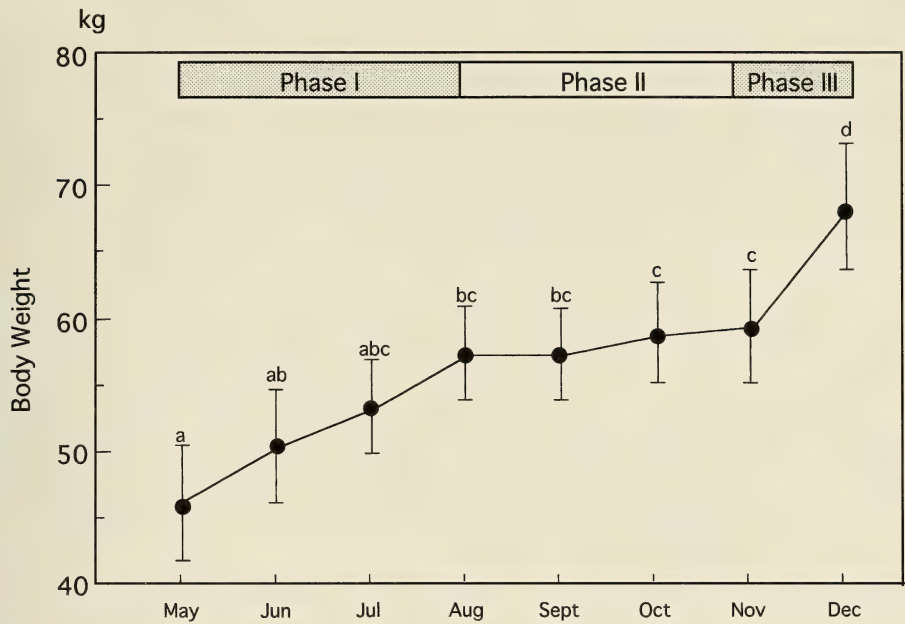
Fig. 1. Monthly body weight change of female captive Asiatic black bears. Vertical bars represent *SD*. Different letters mean significant differences (*P*<0.05).

tion to r_w (Fig. 1). During Phase I, r_ws ranged between 5% and 10%, during Phase II r_ws varied less than 5%, and during Phase III r_ws varied by more than 10% (Table 1).

During Phase I, mean body weight increased gradually from May (46.2±4.4 kg, n=6) to August (57.3±3.5 kg, n=6). Although these month by month changes were not significant, the overall difference between May and August was significant (*P*<0.01). We believe, however, that small sample sizes and large variations among individuals have masked a real month by month increase from May to August, because each individual showed a similar pattern of increasing body weight (Appendix 1). From August to November, mean body weights were stable (59.4±4.3 kg, n=4). The differences between months were not significant during this period (*P*>0.9 for each comparison). In contrast, body weights increased rapidly during November and December (68.4±4.7 kg, n=5), and December body weights were significantly heavier than in November (*P*<0.05).

Table 1. Body weight and the rate of weight increase (r_w) of seven captive female Japanese black bears.

	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Average (kg)*	46.2[a]	50.4[ab]	53.3[abc]	57.3[bc]	57.3[bc]	58.8[c]	59.4[c]	68.4[d]
SD	4.4	4.2	3.6	3.5	3.5	3.8	4.3	4.7
r_w (%)**	—	9.2	5.8	7.5	−0.5	2.7	0.9	15.2

←	Phase I	→	←	Phase II	→	← Phase III →

* Values in the same row followed by different letters are significantly different (*P*<0.05).

** Rate of Weight Increase $= \dfrac{\text{mean body weight} - \text{mean body weight of last month}}{\text{mean body weight of last month}} \times 100$

Discussion

The body weights of seven captive female Japanese black bears increased gradually during spring (Phase I), were stable during summer (Phase II) and increased rapidly in autumn (Phase III). These changes were presumed to be related to annual changes in growth and to seasonal changes in nutritional conditions. Because the body weights of the bears did not differ between years, they were assumed to have been old enough to cease growing. We conclude, therefore, that the weight changes of the bears examined were seasonal.

Seasonal changes in the nutritional conditions of animals are closely related to their physiology. Nelson et al. (1983) defined four seasonal physiological stages in American black bears and grizzly bears, *U. arctos*: hibernation, walking hibernation, normal activity and hyperphagia. In our study of the Japanese black bears, we have shown that body weights change during the stages of normal activity and hyperphagia. Phases I and II appear to correspond to Nelson et al.'s (1983) stage of normal activity, and our Phase III to their hyperphagia stage. The fact that the normal activity stage may be divided into two phases was first recognized in the present study. Further study is required to elucidate the physiological mechanisms separating Phases I and II.

It is impossible to undertake the same kind of continuous study of the nutritional conditions of wild bears as can be done in captivity. Nevertheless, some data on seasonal changes in nutritional conditions of wild Japanese black bears from the post denning period to the active period have been collected (Hazumi et al. 1985; Gifu Prefecture 1995). These data, though not presented statistically, showed that nutritional condition as measured by levels of marrow fat (Hazumi et al. 1985) and of kidney fat (Gifu Prefecture 1995) declined from the post denning period (April to June) to the active period (July to September). These results were opposite to those of the present study that revealed a gradual increase in the body weights from May to August.

Also in contrast to our study are the results of Hellgren et al. (1989) who found that in Virginia and North Carolina where environmental conditions such as day length, temperature and vegetation are similar to those in Japan, the body weights of wild American black bears decreased from early summer (mid June to July) to late summer (August to September). In the wild, poor nutritional condition may result from food shortages from early to late summer, whereas the body weights of captive bears increase because of the availability of sufficient food and because of their lower energy expenditure during this season.

During the pre-denning period, the nutritional condition of wild Japanese black bears seems to improve (Gifu Prefecture 1995). In this study, body weight increased rapidly during Phase III. During the period of hyperphagia, the American black bears increase both their food intake (Nelson et al. 1983) and digestion (Brody and Pelton 1988), both of which are thought to be adaptive for storing energy prior to denning. In our study, both factors might have contributed to the increases in body weight.

Although body weights tended to increase during a single year (Fig. 1), when the same months were compared between different years, they did not differ. This indicates that body weight decreases during winter. Body weight loss during hibernation has been reported for captive American black bears (Watts et al. 1981; Watts and Cuyler 1988; Farley and Robbins 1995). Thus, it is probable that captive adult female bears lose weights during winter, and it is plausible that bears repeat an annual cycle characterized by spring recovery, autumn

increase and winter weight loss while denning.

Two methodological issues have been raised by this study, which require further study. Firstly, in order to obtain weight data, we immobilized the bears. This is, however, not good for their health, and it is also costly. In future, therefore, it is recommended that a method not requiring immobilization be used. Secondly, as we did not measure the amount of food, we were unable to relate food availability to changes in body weight. Studies under controlled conditions are needed for a better understanding of the food-body weight relationship in the Asiatic back bear.

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Watts, P. D., Oritsland, N. A. and Ronald, K. 1981. Mammalian hibernation and the oxygen consumption of a denning black bear (*Ursus americanus*). Comparative Biochemistry and Physiology 69: 121–123.

Appendix 1. Body weights (kg) of seven captive female Japanese black bears.

Bear	1994 Oct.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
W31		44.0	53.0	57.0	61.0	60.5	59.0	—	70.5
W32	57.0	—	45.0	48.0	52.0	51.5	53.0	58.0	60.0
W33	64.0	49.0	52.0	56.0	—	59.0	63.0	—	71.0
W34	54.0	41.0	47.0	50.0	55.0	54.0	56.0	54.0	—
W35	65.0	50.0	55.0	—	61.0	60.0	62.0	—	70.0
Y45	59.0*	42.0	—	54.0	58.0	60.0	—	62.0	—
Y48		51.0	—	55.0	57.0	56.0	60.0	63.5	70.5
Mean	60.5	46.2	50.4	53.3	57.3	57.3	58.8	59.4	68.4
SD	5.1	4.4	4.2	3.6	3.5	3.5	3.8	4.3	4.7

* weighed in September.

The distribution and habitat use of the Eurasian red squirrel *Sciurus vulgaris* L. during summer, in Nopporo Forest Park, Hokkaido

Tsung Hung Lee[1] and Hiromi Fukuda[2]

[1,2]*Graduate School of Environment Earth Science, Hokkaido University, North 10 West 5, Sapporo 060-0808, Japan*

Abstract.   The distribution and habitat use of the Eurasian red squirrel *Sciurus vulgaris* L. was studied in Nopporo Forest Park (43°20′ N, 141°30′ E), Hokkaido, Japan where a study area consisting of a total of 401, 200 m by 200 m, grid squares was established.   Observations were made of individuals, their dreys, their feeding signs, and their footprints (after dusting with wheat flour).   Squirrels were found to be widely distributed throughout the study area, and to inhabit 45 of the 401 squares (11.2%).   Squirrels occurred at high frequencies in three areas within the forest.   The percentages of squares in which squirrels lived, differed significantly between different forest types, with 28.2% of squares in evergreen coniferous forest used, 5.3% in deciduous coniferous forest; 5.2% in mixed forest; 3.9% in deciduous broad-leaved forest, and 0% in other areas.   We concluded that dusting with flour was a useful method for revealing footprints, and that this facilitated the ease study of squirrel distribution.   The distribution of the red squirrel clearly depends on the forest type.   Coniferous forest areas were selected as habitat by squirrels during summer because they provided good sources of food and ideal sites for building dreys.

Key words: distribution pattern, footprints revealed with flour, forest type, habitat use, *Sciurus vulgaris*.

The Eurasian red squirrel, *Sciurus vulgaris* L., is widespread throughout Hokkaido, Japan (Environment Agency 1983; Takaragawa 1996).   Significant changes to the landscape, as a result of human activities such as deforestation for agriculture and/or urbanization, have, over the last century, cause a drastic reduction in the area of suitable forest habitat, which has led to the isolation of squirrel populations.   Such isolated populations are at risk from the negative effects of isolation such as inbreeding depression (Wildt et al. 1987; Brewer et al. 1990) which may lead to the extinction of populations or even species.   In England, where the distribution range of the red squirrel has reduced and fragmented in this century, areas of forest of more than 2,000 ha are now deemed necessary if they are to serve effectively as red squirrel reserves (Gurnell and Pepper 1993).

Information on the local distribution and abundance of animal population is an important aspect of management-oriented investigations of wildlife.   To that end, several cen-

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[1]*Present address: Rm. 2, 3rd Fl., 25, Lane 238, Szeping R., Taichung 406, Taiwan;   E-mail: tsunghun@ms29.hinet.net*

sus techniques have been evaluated for the red squirrel, including elaborate capture-recapture programs (Moller 1986; Wauters and Dhondt 1990b); drey counts (Tittensor 1970; Wauters and Dhondt 1988, 1990a; Van Apeldoorn et al. 1994); and counting tracks and food remains (Andrén and Lemmell 1992; Kadosaki 1995).   For this study we used a combination of direct observations of individuals, and observations of dreys, feeding signs, and footprints dusted with wheat flour, in order to study the distribution and habitat use of the Eurasian red squirrel in Nopporo Forest Park during the summer from June to September 1996.

Study area

Research was conducted in Nopporo Forest Park (43°20′N, 141°30′E), which is situated in west Hokkaido, 11–15 km east of central Sapporo.   Nopporo Forest Park has an area of 2,051 ha, and stretches over parts of Sapporo, Ebetsu and Kitahiroshima administrative areas.   The forest is designated as a natural recreational forest, and the whole area lies within a wildlife protection area.   Nopporo forest has become completely isolated from other areas of lowland forest as a result of the spread of both agriculture and urbanization. The forest now consists mainly of natural deciduous forest, but has some areas of planted conifers within it.   The total area of our study, after excluding bogs, lakes, and buildings, amounted to about 1,611 ha.

Materials and methods

This study was processed in three stages from 1 June to 15 September 1996.

During Stage I, from 1 June to 20 June 1996, a study area of 16 ha was established and searched for squirrels, their dreys and their feeding signs, in order to obtain basic habitat utilization information.

During Stage II, from 25 June to 15 July 1996, the following program was followed in order to evaluate a method for studying squirrel distribution and habitat use.   1) A square covering about 1 ha (100 m × 100 m) was establish.   2) In the center of this area, a feeding station with a radius of 60 cm was establish on the ground.   Approximately 300 g of wheat flour was spread in the middle of the feeding station covering an area with a radius of 25 cm. In addition, one walnut, one acorn, one peanut, one pistachio and ten sunflower seeds were put on the feeding station for a whole day in order to attract squirrels to the area covered with flour.   3) When squirrel, or other animals, visited the feeding station they picked up flour on their feet, and left trails of footprints as they left.   The species leaving footprints could be identified on the basis of the shape of the hind foot and on the stride length.   4) In order to distinguish between the individuals leaving footprints, we observed them with binoculars (×7) at a distance of 20 m from the feeding station.   We were able to identify three squirrels individually on the basis of their coat color, their size, the cuts of their ears, and by the size of the footprints.   5) The day-range of the three known individuals was established by setting eight new feeding stations to the north, south, east, and west of the main feeding station and at distances of 100 m and 200 m away, in order to see whether the same individual also visited there or not.   All three squirrels visited the new feeding stations set 100 m away from the original site, but none visited feeders set 200 m away.   Therefore, the home ranges of these squirrels were equal to or less than the size of the grid (200 m × 200 m).

During Stage III, from 16 July to 15 September 1996, we investigated the distribution of squirrels and their habitat use throughout the whole forest area. First we established 401, 200 m by 200 m (4 ha), grid squares in the study area. Each square was investigated every two days. On the first day the feeding station was provisioned, and on the second day footprints were followed. We also looked for nests, recording nesting tree details, searched for feeding signs, and watched for individuals within a 50 m radius of the feeding station. The dominant tree species within a 50 m radius of the feeding station were also noted. We also investigated the distribution of squirrels (using the methods described in stage III above) in four areas adjoining the main study area. These areas were: the Hokkaido Forest Tree Breeding Institute (including the gene reservation region); Nopporo Prefectural General Sports Park; the Historical Village of Hokkaido, and the campus of Rakuno Gakuen University (Fig. 1).

Results

Distribution pattern

Eurasian red squirrels were found (on the basis of direct observation, or of finding their footprints, their dreys or their feeding signs) in 45 (11.2%) of the 401 study squares. Occupied squares were scattered around the study area. When the shortest distance between squares being used by squirrels was equal to or less than 400 m, we drew minimum convex polygons and so were able to recognize three areas (A, B, and C) where squirrels were concentrated. Area A consisted of 18 squares (18/45; 40%); Area B consisted of 13 squares (29%), and area C consisted of six squares (13%; see Fig. 1). In area A, squirrels were scattered throughout a large area, and in area C they were scattered over a smaller area, whereas in area B they were highly concentrated.

From additional research outside the main study area, in the Hokkaido Forest Tree Breeding Institute, squirrels were found in four squares, and their distribution was concentrated in the gene reservation section (the distribution of squirrels in the three other areas can be seen in Fig. 1).

A total of 25 individual squirrels was observed, in the 401 squares of the study area; 11 individuals were identified in area A; nine in area B; three in area C; and two in other areas. We are confident that observations were of different individual squirrels because either different squirrels were seen in the same squares simultaneously or they were seen in squares separated by more than 400 m. We concluded, therefore, that the squirrels in Nopporo Forest Park were mainly distributed in area A, B and C during summer.

Squirrels were more often found in some tree species than in others, so for example, twelve were seen on *Abies sachalinensis*; six on *Pinus strobus*; four on *Larix leptolepis*; two on *Picea glehnii*, and one on *Quercus mongolica*.

A total of 28 dreys was found in just 15 squares, although in one square with a drey we were unable to find either footprints, feeding signs or individuals, suggesting that the drey may have been disused. Feeding signs were found in 24 squares, and in a further square we found feeding signs but were unable to locate individuals, dreys, or footprints. Distinctive feeding signs were recognize on walnuts, chestnuts, acorns and the cones of *A. sachalinensis*, *Pinus koraiensis* and *P. strobus*. Among the total of 45 squares showing signs of squirrels, activity in 43 (95.6%) of them was detected using the floured footprint method.
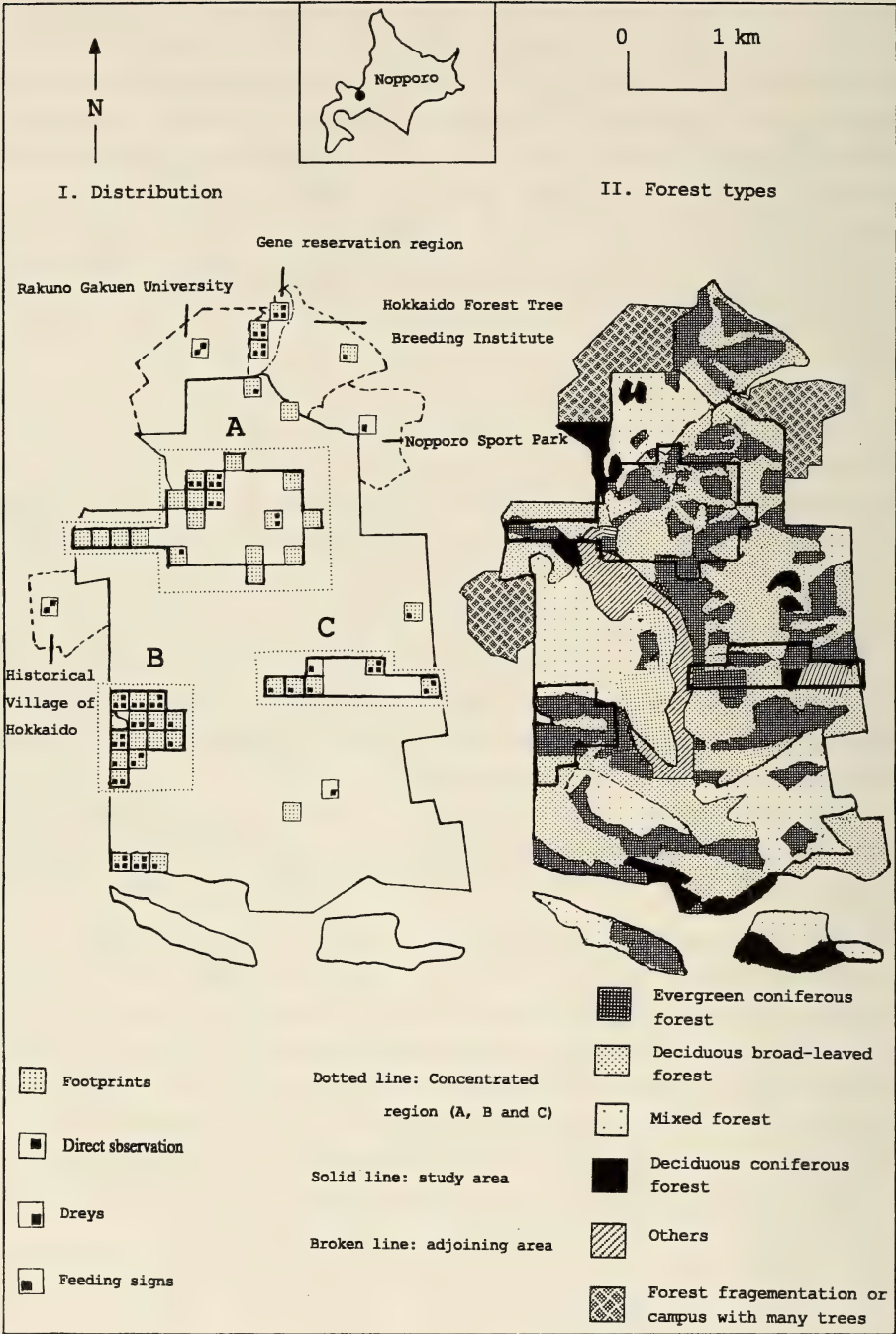
Fig. 1. The distribution pattern of the red squirrel in the Nopporo Forest Park with 200 m grid squares (I. Distribution) and the vegetation of the Nopporo Forest Park and the adjoining areas (II. Forest types).

Habitat use

According to the investigation of the dominant trees near the feeding stations, and on the basis of published vegetation maps (Environment Agency 1981; Sapporo District Forestry Office 1992), the forest vegetation could be divided into five broad types: 1) mixed forest, consisting of a natural community of *Picea jezoensis*, *A. sachalinensis*, *Q. mongolica*, *Tilia japonica* and *Acer mono*; 2) deciduous broad-leaved forest, consisting of a natural community of *A. mono* and *T. japonica*; 3) evergreen coniferous forest, consisting of a plantation community of *A. sachalinensis*, *P. glehnii* and various exotic trees; 4) deciduous coniferous forest, consisting of a plantation of *Larix leptolepis*; and 5) others vegetation types including cultivated meadow, fields and young plantations (see Fig. 1). On the basis of squirrels presence and on the forest community within each square, we were able to examine habitat use in different forest types. Among squares where evergreen coniferous forest predominated 28.2% of squares were occupied by squirrels. Where deciduous coniferous forest was dominant 5.3% of squares were occupied. In mixed forest 5.2% of squares were occupied by squirrels, and in deciduous broad-leaved forest 3.9% of squares were occupied. In the fifth category of vegetation, no squares (0%) were occupied. The differences in forest use were statistically significant ($P<0.001$; Table 1). Squirrels were present in just 4.4% of natural vegetation units, but were found in 25.7% of the squares where the forest consisted of plantations. The difference in the rate of habitat use between natural vegetation and plantations was also significant ($\chi^2=38.4$, $df=1$, $n=387$, $P<0.001$).

In the three areas where squirrels were concentrated, area A region consisted of evergreen coniferous forest, deciduous broad-leaved forest, mixed forest. Area B consisted mainly of evergreen coniferous forest, with some deciduous broad-leaved forest. Area C region consisted mainly of evergreen coniferous plantation, deciduous broad-leaved forest and some planted deciduous conifers. Among these three areas, however, squirrels were mainly distributed in areas with evergreen coniferous forest. Squirrels were less commonly found in areas of deciduous broad-leaved forest, mixed forest, deciduous coniferous forest or others vegetation types. In terms of the total number of squares occupied by squirrels (45), 33 (73.3%) were in evergreen coniferous forest, and 12 (26.7%) were in deciduous coniferous, mixed and deciduous broad-leaved forests. Similarly, in the Hokkaido Forest Tree Breeding Institute, adjoining the study area to the North, squirrels were mainly distributed in the gene reservation region, which consisted of various types of coniferous plantation. More feeding signs (on 13 species) were found here than in other areas.

Further analysis of the distribution of dreys in relation to tree species, indicated that

Table 1. The number of grids with squirrel in different forest types.

Forest type	Grids of presence(%)	Total of grids
Evergreen coniferous forest	33(28.2)	117
Deciduous coniferous forest	1(5.3)	19
Mixed forest	5(5.2)	97
Deciduous broad-leaved forest	6(3.9)	154
Others	0(0)	14
Total	45	401

$\chi^2=50.689$, $n=401$, $df=4$, $P<0.001$

Table 2.   The numbers of the dreys built in different type of trees.

Type of trees	Nopporo Forest Park				Hokkaido Forest Tree Breeding Institute	Total (%)
	A	B	C	Others	Gene reservation region	
Evergreen coniferous trees	10	9	2	4	8	33(89.2%)
Deciduous coniferous trees	0	2	0	0	1	3(8.1%)
Deciduous broad-leaved trees	1	0	0	0	0	1(2.7%)
Total	11	11	2	4	9	37

most dreys (89.2%) were built in evergreen coniferous trees, although some were built in deciduous coniferous trees and some in deciduous broad-leaved trees (Table 2).

Discussion

Tracking footprints is a well-established means of confirming the presence of mammals. Footprints are not always conspicuous, however Kadosaki and Inukai (1995) showed that it was possible to use lime flour to make the footprints of brown bear *Ursus arctos* more conspicuous, and also to aid in their individual identification.   In this study we used a similar method, imprinting the footprints of Eurasian red squirrels with wheat flour, in order to facilitate tracking and censussing them.   This method enabled us to confirm that as squirrels will visit feeding stations up to 100 m apart, but not 200 m apart (see stage II above), then 200 m × 200 m grid squares can be used to study the distribution of the squirrels in Hokkaido.

Seasonal and annual changes in habitat condition are known to affect the distribution (Gurnell 1983), and the home ranges of males are known to be larger during the breeding season (Wauters and Dhondt 1992).   We conducted our study therefore during the three months of summer when squirrels density was likely to be highest (Takaragawa 1980; Gurnell 1983), and used direct observation, flour print tracking, observations of dreys, and feeding signs, in order to investigate the distribution pattern and habitat use of squirrels.   During this study, the presence of the footprints (in 43 squares) proved to be the best evidence of the presence of squirrels in a given area, with direct observation of squirrels (in 11 squares) being a somewhat less effective method of study them.   The presence of footprints reflects activity well, and as squirrels came to each feeding station voluntarily and without being disturbed, their subsequent movements were considered to be natural.

Although red squirrels use two types of nests, spherical shaped nests built amongst tree branches, and dens in tree-holes lined with nest material (Tittensor 1970; Wauters and Dhondt 1990a), dreys are generally the commonest form of nest in both coniferous and deciduous woodlands (Wauters and Dhondt 1990a).   After the breeding season, and hence during summer, dens are less important (Tittensor 1970), therefore, although we used dreys as one means of confirming the distribution pattern of squirrels, we disregarded misshapen dreys as it was unlikely that they were still in use.

The use of four different types of field signs enabled us to study the distribution pattern of squirrels, though different method had widely differing degrees of success.   The number of grid squares where squirrels were detected on the basis of these different field signs was: 43

for footprints > 24 for feeding signs > 15 for dreys > 11 for direct observation of individuals. Squirrels were detected in 17 squares purely on the basis of tracing their footprints. Their presence in one square was confirmed only by the finding of a drey, and in another by the finding of feeding signs. Sightings of individuals were only made in conjunction with other signs, and their presence was not confirmed in any squares by direct observation alone. We suggest, therefore, that tracking footprints dusted in flour is a very effective method for the study of the distribution and movements of the red squirrels.

As in Europe, many woodlands in Japan have become fragmented, so that where large red squirrel populations used to occur, they have now been reduced to small populations in isolated parts of their old range (Celada et al. 1994; Van Apeldoorn et al. 1994; Wauters et al. 1994). Habitat fragmentation has been shown to have impacts on both the distribution and probability of occurrence of this species (Van Apeldoorn et al. 1994), and it has been recommended that forests larger than 2,000 ha are necessary as red squirrel reserves (Gurnell and Pepper 1993).

In this study, the widespread distribution, with some areas of concentration, indicate that squirrels are actively selecting their habitat. Additional research using radio-telemetry showed that during the mating season, from February to June, males living in area A also temporarily visited the Hokkaido Forest Tree Breeding Institute, while males from the adjoining areas (Hokkaido Forest Tree Breeding Institute, Nopporo Prefectural General Sports Park, the Historic Village of Hokkaido and the campus of Rakuno Gakuen University) also sometimes visited area A (Lee and Fukuda unpublished data). Thus the squirrels living in these adjacent areas are also considered to belong to the same population as in Nopporo Forest Park. In this area, totalling more than 2,000 ha of forest, red squirrels were considered to be actively selecting their habitat and concentrating in three high density areas. During the mating season, however, males travel to other areas in order to mate, and hence avoid the negative effects of reduced genetic variation (Wauters et al. 1994), and habitat loss by the forest fragmentation (Andrén and Delin 1994). Whereas red squirrels were found readily in the larger area of forest in Nopporo, none were found in small isolated urban habitats such as on the Hokkaido University campus (182 ha) or in the Botanical Garden (20 ha), despite there being plentiful food resources in the form of walnut, acorns, and pine cones. Their absence may have been because the areas of habitat were too small for them, or because of the presence of too many predators such as cats and/or crows.

The coniferous community of the Nopporo Forest Park, produced by frequent cuttings and by wind damage, has been maintained by planting *A. sachalinensis*, and *P. jezoensis*, *L. leptolepis* and exotic trees such as *Pinus koraiensis*, and *P. strobus*. The natural mixed forest community consisted mainly of *Picea jezoensis*, *A. sachalinensis*, *T. japonica* and *Acer mono*, while the broad-leaved forest consisted mainly of *A. mono* and *T. japonica*. Although, *Juglans ailanthifolia* was also present, it was scarce (Tatewaki and Igarashi 1973).

Eurasian red squirrels feed on a wide range of different food types, however tree seeds form the most important part of their diet (Moller 1983; Gurnell 1987; Wauters et al. 1992), and coniferous tree seeds can provide food year round (Wauters et al. 1992). Stomach contents analysis has also shown that pine seeds are the most important component of their diet (Gronwall and Pehrson 1984). In our study, red squirrels occurred more commonly in evergreen coniferous forest than in either broad-leaved forest or mixed forest. Moreover, red squirrels were also abundant in the part of the gene reservation region of the Hokkaido

Forest Tree Breeding Institute where a wide range of coniferous tree species occur, suggesting that habitat selection may be made on the basis of the availability of good food resources.

The concentration of squirrels in three regions of Nopporo Forest Park is probably due to habitat selection on the basis of good food resources and good locations for building dreys. Furthermore, habitat selection may be one aspect of an adaptive breeding strategy, if more squirrels can live in the same area, then perhaps their chances of finding a partner are increased.

In conclude, tracking red squirrel footprints using flour is a very helpful method for studying their distribution and their habits. Observations made on these basis indicate that the red squirrel in Hokkaido selects coniferous forest habitats in summer because they provide good sources of food and good sites to build dreys. From the standpoints of conservation of this species, maintaining large areas of forest which include or are connected to areas of coniferous forests are very important. Further studies on impact of environmental factors on squirrels, such as seasonal changes in habitat quality, predator distribution, and the significance of forest floor vegetation (especially the presence of bamboo grass) are needed.

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Molar wear rates in Sika deer during three population phases: increasing versus decline and post-decline phases

Hiroshi Takahashi[1], Koichi Kaji[2] and Toru Koizumi[3]

[1]*Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060–0810, Japan*
[2]*Hokkaido Institute of Environmental Sciences, Sapporo 060–0819, Japan*
[3]*Kyushu Research Center, Forestry and Forest Products Research Institute, Kumamoto 860–0862, Japan*

Abstract. Wear rates of lower first molars (M_1) in Sika deer, *Cervus nippon*, were compared among the increasing, declining and post-decline phases of population dynamics on Nakanoshima Island, Hokkaido, Japan, to evaluate the effects of food limitations on deer feeding ecology. Teeth specimens were collected also from a population in eastern Hokkaido, as a control, where foods were abundant. The maximum length and width of M_1 were not different among the three phases. A linear regression coefficient for log-transformed M_1 height against age was not different between males and females, but significantly smaller in the post-decline phase population than in the increasing phase and the control populations. The results suggest that M_1 wear rates increased as food declined.

Key words: *Cervus nippon*, food limitation, molar wear rates, Nakanoshima Island, population phase.

Food limitation influences life history characters of mammals through its effects on their physical conditions (Fowler 1987). Since tooth wear of ungulates reflects food quality (Morris 1972; Fortelius 1985) and affects physical condition by its influence on ingestion and mastication (Skogland 1988), tooth wear rate can be used as an index of life history characters. For example, female reindeer, *Rangifer tarandus*, in a low density population showed lower wear rates of molars than in a high density population (Skogland 1988). Similarly, tooth wear was related to survival rates of roe deer, *Capreolus capreolus* (Gaillard et al. 1993) and kudu, *Tragelaphus strepsiceros* (Owen-Smith 1993). These results suggest that tooth wear patterns are related to the demography of ungulate species.

    Three individuals of Sika deer, *Cervus nippon*, were introduced to Nakanoshima Island in Lake Toya, Hokkaido, Japan from 1957 to 1965 (Kaji et al. 1988). The population increased to 299 (57.5/km²) in 1983, and grazing effects on vegetation became apparent (Kaji et al. 1991). The population crashed under food limitations in 1984, and gradually recovered to approximately 180 (34.6/km²) in recent years (Hokkaido Institute of Environmental Sciences, hereafter HIES 1997). The dynamics of this population and its impacts on vegetation have followed the typical pattern of introduced ungulates (Caughley 1970). We had

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[1]*E-mail: yuji@ees.hokudai.ac.jp*

the opportunity to test the influence of food limitations on tooth wear rates during different population dynamic phases. Since life history parameters such as fecundity decreased as food availability declined (Kaji et al. 1988), tooth wear rates may also be an indicator of population quality if they vary with population phases.

Materials and methods

Tooth samples were collected from naturally dead deer on Nakanoshima Island from 1980 to 1984, and from 1992 to 1997. These were divided into the increasing (1982 or before), the decline (1983 and 1984) and the post-decline (1985 or after) phase groups according to the year of death for each sample (hereafter INC, DEC and POST, respectively). A control group (CONT) was sampled from the deer killed in pest controls in 1990 in eastern Hokkaido, where the population showed high fat reserves (Yokoyama et al. 1996) and high reproductive rates (Suzuki and Ohtaishi 1993), which indicate good nutrition.

Age of each sample was determined by cementum annuli from the first incisors or the first molars after Ohtaishi (1980). The lower first molar (M_1) was used to measure wear rates because it has the longest exposure to wear. Morris (1972) recommended measuring the area of exposed dentine to assess tooth wear because of ever-increasing quantity. However, in many of our samples, wear extended over the entire occlusal area, and the area of dentine exposed reached the ceiling. Therefore, we measured the crest height from the cervical line to the postloph lingual crest (Ohtaishi 1980) to the nearest 0.01 mm with a caliper. The maximum M_1 length and width were also measured for samples from Nakanoshima Island in order to compare M_1 size among the different phases.

We obtained samples ten years old and older from the POST, but not from other phases. To compare wear rates in equivalent age classes, only samples under ten years of age were used in the analysis. The M_1 length and width were compared between sexes and among the phases by two-way ANOVA. Linear regression coefficients for the log-transformed crest height against age were compared between sexes and across phases. If ANOVA for parallelism of regression lines proved significant, multiple comparisons of regression coefficients were carried out using the Tukey-Kramer method (Sokal and Rohlf 1995).

Results

The means of the maximum M_1 length and width of the three phases are shown in Table 1. Both the length and width were significantly different between sexes (two-way ANOVA:

Table 1. Mean ($\pm SD$) of maximum M_1 length and width by sex in each phase.

Phase	Sex	n	Length (mm)	Width (mm)
Increasing	Female	6	16.14±0.28	11.09±0.61
	Male	7	16.37±0.65	11.56±0.33
Decline	Female	18	16.28±0.64	11.39±0.40
	Male	30	16.33±0.56	11.49±0.44
Post-decline	Female	22	16.12±0.54	11.14±0.60
	Male	14	16.66±0.43	11.44±0.45

length, $F_{1, 91}=4.013$, $P=0.048$; width, $F_{1, 91}=5.459$, $P=0.022$), but not among phases (length, $F_{2, 91}=0.641$, $P=0.529$; width, $F_{2, 91}=1.265$, $P=0.287$), and sex-phase interaction was not significant (length, $F_{2, 91}=1.796$, $P=0.172$; width, $F_{2, 91}=0.807$, $P=0.449$). This suggests that M_1 size is a sexually dimorphic character unrelated to food limitation.
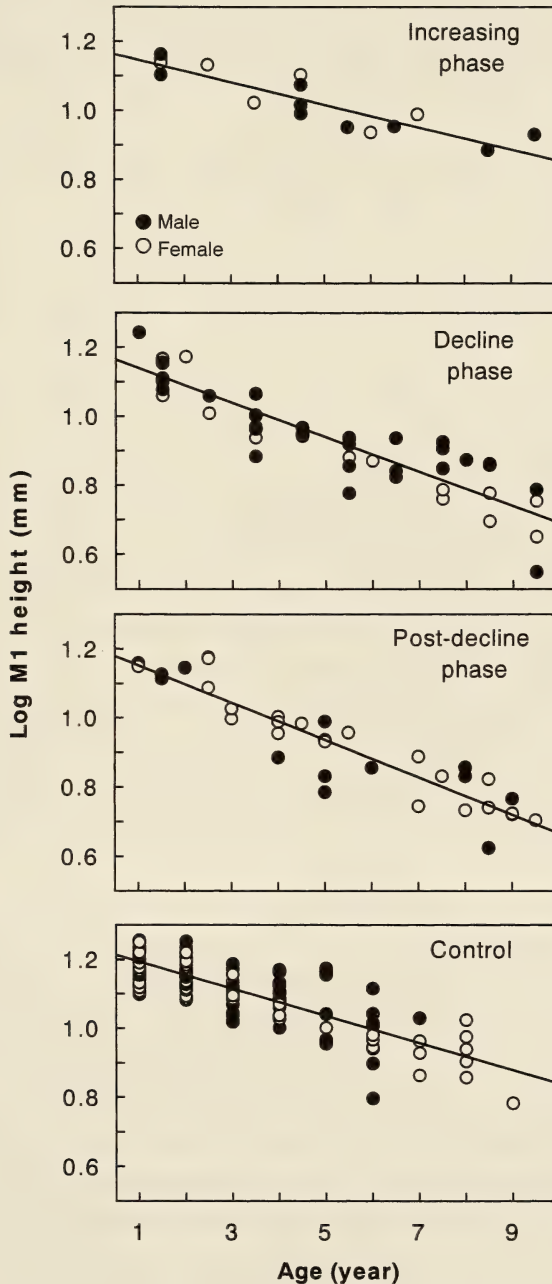

Fig. 1. Changes in log-transformed M_1 height with age in Sika deer during three population phases on Nakano-shima Island and in deer from eastern Hokkaido (control).

Table 2.   Regression coefficients for log M_1 height against age (b) by sex in each sample group.

Sample group	Female				Male				Sexual difference
	n	b	SE	P	n	b	SE	P	
Increasing phase	7	−0.033	0.009	*	10	−0.030	0.005	**	*ns*
Decline phase	18	−0.053	0.004	**	30	−0.047	0.005	**	*ns*
Post-decline phase	22	−0.053	0.004	**	14	−0.056	0.008	**	*ns*
Control	55	−0.040	0.002	**	109	−0.036	0.004	**	*ns*

P (two-tailed): *, <0.05;   **, <0.001.

Table 3.   Regression coefficients for log M_1 height against age (b) in each sample group containing both sexes.

Sample group	n	b	SE	P
Increasing phase	17	−0.032	0.004	<0.001
Decline phase	48	−0.050	0.003	<0.001
Post-decline phase	36	−0.054	0.004	<0.001
Control	164	−0.039	0.002	<0.001

Changes in log-transformed crest height with age are shown in Fig. 1.   Sexual differences in regression coefficients of log M_1 height against age were not detected within each phase group nor in the mainland control group (ANOVA for parallelism of regression lines: $F_{1, 8}$ $=0.083$, $P=0.219$ for INC; $F_{1, 16}=0.605$, $P=0.448$ for DEC; $F_{1, 19}=0.066$, $P=0.199$ for POST; $F_{1, 12}=0.553$, $P=0.472$ for CONT; Table 2).   Therefore, data of both sexes were pooled to increase sample sizes, and regression coefficients were recalculated (Table 3). ANOVA detected significant differences among the three phases ($F_{3, 40}=4.880$, $P=0.006$), and multiple comparison showed significant differences between INC and POST ($P<0.05$) and between POST and CONT ($P<0.05$).   These results suggest that wear rates of M_1 in INC were equivalent to those in CONT, and that the wear rates in POST were higher than those in INC.

Discussion

On Nakanoshima Island, food resources were abundant in 1980, but the deer began to consume more tree bark as palatable plants declined in 1982 and 1983 (Kaji et al. 1991). Thereafter, production in short-grass communities during summer decreased from 198.6 g/m² in 1984 to 63.9 g/m² (average, 1992–1994, Miyaki et al. 1995).   Available understory plants were scarce and the deer were obliged to consume short greens and fallen leaves.   The production of fallen leaves was estimated to be 28.7 kg/ha/month in July 1994, while that of the short-grass communities was simultaneously estimated to be 144.4 kg/ha/month (Miyaki et al. 1995).   However, due to the limited area of short-grass communities (0.4% of the island), and the fact that deer can use them only during the snow-free season (late April to October), the short greens could support only 29 deer (14% of the population) even in summer, and the deer would depend mostly on fallen leaves (Miyaki et al. 1995).   Thus, high quality forage was not available in either DEC which experienced sudden food shortage, or

in POST which faced continuous food limitations. Consequently, wear rates in DEC were not apparently different from INC, while those in POST were significantly higher than in INC and CONT. In addition to experiencing changes in food quality, deer were often observed to ingest soil when grazing. Similar reports have been made of higher tooth wear rates associated with soil ingestion by white-tailed deer, *Odocoileus virginianus* (Rue 1978) and with the presence of abrasive elements in the diet of Spanish ibex, *Capra pyrenaica* (Fandos et al. 1993). Previous studies demonstrated that food limited populations showed higher wear rates than food rich populations in reindeer (Skogland 1988) and Spanish ibex (Fandos et al. 1993). In this study, we found that a deer population under food limitations showed higher wear rates than when under food rich conditions.

On Nakanoshima Island, body size of Sika deer and degree of sexual dimorphism were reduced by the effects of food limitation (Kaji et al. 1988). In this study, however, M_1 sizes (length and width) were different between sexes but not among the three phase groups. Since M_1 reaches a given size before skeletal growth is completed (Fortelius 1985), M_1 sizes may not be as influenced by food limitation as skeletal sizes are. Tooth size appears not to affect wear rates.

Some studies have suggested that higher teeth wear rates in males are a result of the male's greater food consumption associated with larger body size (black-tailed deer, *Odocoileus hemionus columbianus*, Thomas and Bandy 1975; Sika deer, Ohtaishi 1976; Takatsuki 1998), although statistically significant differences were either unexamined or equivocal. Takatsuki (1998) showed that wear rates of first incisor were higher in male sika deer than in females on Kinkazan Island, where males consumed foods of lower quality, but not obviously different in Mt. Goyo, where higher quality foods were available to both sexes. Since tooth wear reflects diets of animals for a given period, sexual differences in wear rates might arise when sexual differences in food habits occur and continue for certain periods. Under food rich conditions, high quality foods may be consumed by both males and females. On the other hand, under the food limitations on Nakanoshima Island, both sexes were forced to consume low quality foods. These situations would make sexual differences in food habits unclear, consequently obscuring sexual differences in wear rates. In addition, the patterns of sexual differences in molar wear may differ from that in incisor wear.

The results of the present study rely on the accuracy of age estimation. McCullough (1996) pointed out that the cementum aging technique in white-tailed deer sometimes failed because the cementum annuli disappeared when a population density was low and available forage was relatively abundant. If our age estimates are similarly biased, older animals of INC and DEC would have had more years during times of relative food abundance. Similarly, samples of the CONT were collected in 1990 when the deer were well nourished irrespective of age (Yokoyama et al. 1996). In these cases, since the regression slopes may become less steep in INC and DEC or be unchanged in CONT, differences between regression slopes of INC and POST would be even greater. Thus, our conclusion that wear rates increased substantially during the post-decline phase would not be affected greatly by underestimates of age.

In conclusion, both the duration and the extent of food limitations clearly contribute to differences in tooth wear rates. Tooth wear seems to be not only population-specific as Fandos et al. (1993) suggested, but also population phase-specific. Tooth wear rates could

be a relative indicator of population health and food conditions.

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Genetic relationships within and between the Japanese marten *Martes melampus* and the sable *M. zibellina*, based on variation of mitochondrial DNA and nuclear ribosomal DNA

Tetsuji Hosoda[1], Hitoshi Suzuki[2], Masahiro A. Iwasa[3], Mitsuhiro Hayashida[4], Shigeki Watanabe[5], Masaya Tatara[6] and Kimiyuki Tsuchiya[7]

[1]*Gobo Shoko High School, 43-1 Komatsubara, Yukawa, Gobo 644-0012, Japan*
[2,3]*Graduate School of Environmental Earth Science, Hokkaido University, North 10, West 5, Kita-ku, Sapporo 060-0810, Japan*
[4]*Faculty of Agriculture, Yamagata University, Tsuruoka 997-8555, Japan*
[5]*2-24-5 Ao-madanihigashi, Mino 562-0022, Japan*
[6]*Wildlife Conservation Centre of Tsushima, Nature Conservation Bureau, Environment Agency, Saozaki, Kami-agata, Nagasaki 817-1600, Japan*
[7]*Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki 889-1692, Japan*

Abstract.   We examined the intra- and inter-specific genetic relationships of the Japanese marten *Martes melampus* and the sable *M. zibellina* using cytoplasmic and nuclear DNA markers.   The interspecific sequence divergences in the 402 base pairs of the mitochondrial cytochrome *b* gene averaged 3.3%.   The extent of the divergences among thirteen individuals of *M. melampus* collected from Honshu, Shikoku, Kyushu and Tsushima was small ($\leq 0.5\%$), irrespective of their fur color variation.   A somewhat higher degree of intra-specific variation (up to 1.3%) was observed among *M. zibellina* specimens, but the extent of inter-populational variation between Primorye, Russia, and Hokkaido, Japan, was not so high (minimum 0.2%), suggesting that there has been recent genetic communication between Hokkaido and the continent.   Among the 24 restriction sites of the nuclear ribosomal DNA spacer, there was no difference within either species, however one site differed between the two species.   Using these molecular markers we confirmed that an animal from Hokkaido, showing the typical morphological characteristics of *M. melampus*, possessed the same genotype as *M. melampus* from Honshu.   From these results and descriptions in the literature, we presumed that the animal in question could be a descendent of *M. melampus* introduced to Hokkaido from Honshu by fur farmers about 50 years earlier.   Eight animals examined from Hokkaido showed no indication of hybridization between the two species.

Key words: Japanese marten *Martes melampus*, sable *Martes zibellina*, geographic variation, mitochondrial cytochrome *b*, ribosomal DNA.

Two species of martens, the sable *Martes zibellina*, and the Japanese marten *M. melampus*, occur naturally in the Japanese archipelago.   The sable ranges across the northern part of

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the Eurasian Continent, and the population occurring on Hokkaido, Japan, is described as the endemic subspecies *M. z. brachyura*. *Martes melampus* occurs in the main Japanese archipelago, southern Hokkaido, and the Korean Peninsula. Three separate subspecies are recognized on the basis of differences in their coat coloration (Anderson 1970; Corbet 1978): *M. m. melampus* on Honshu, Shikoku, and Kyushu; *M. m. tsuensis* on Tsushima Island; *M. m. coreensis* on the Korean Peninsula (although the reliability of its identity is still controversial). *Martes zibellina* shows within population, regional, and seasonal variation in fur color and quality (Ognev 1931; Stroganov 1962; Anderson 1970). In winter, the sable's fur color ranges from light yellow or grayish brown, often with a russet tinge, to dark blackish-brown. A very dark and silky fur characterizes the sables occurring in Transbaikalia and southern Yakutia, Russia, while from the west to east the color gradually becomes paler, and the fur becomes coarser. In general, however, there is considerable fur color variations within populations (Stroganov 1962).

Martes zibellina of Hokkaido also shows fur color variation, from pale yellow, to cork colored, and to dark brown (Imaizumi 1986). The color of those with yellowish fur is indistinguishable from yellow specimens of *M. melampus* from northern Honshu. *Martes melampus* from Kyushu and Honshu (except for the southern part of the Kii Peninsula), having body fur which changes from dark brown to yellow in winter, tends to show some regional variation in fur color (Hosoda and Oshima 1993). The head remains light gray, and the legs are dark brown. Individuals from the the Tohoku region of northern Honshu are a particularly vivid yellow. In contrast, individuals from the southern Kii Peninsula, from Shikoku and Tsushima, remain dark brown throughout the winter, although the throat is either yellow or pale yellow (Hosoda and Oshima 1993). The evolutionary history of these two closely related species remains largely unknown, and no evaluations of genetic differentiation among the regional populations based on fur-color variation have been made.

Martes melampus have been introduced to various parts of Japan where this species did not occur naturally, in particular to Sado Island and Hokkaido. Just before World War II, *M. melampus* was introduced from the Tohoku region to a breeding farm near Sapporo, Hokkaido. When feed became unavailable during the war, the animals were released into the wild (Inukai 1975). That they and their descendants survived, and are still being observed in southern Hokkaido has been noted by Inukai (1975) and Kadosaki (1996). Given that the two species are thought to have a similar chromosomal constitution, with the same diploid number and fundamental arm number, then hybridization may have occurred or be occurring between the Hokkaido native *M. z. brachyura* and introduced *M. melampus* (Tsuchiya 1979; Obara 1982, 1991; Tsuchiya unpublished). Natural hybrid between the two sympatric *Martes* species, *M. zibellina* and the pine marten *M. martes* in the Pechora Basin and the trans-Urals of Russia, have been reported (Ognev 1931; Novikov 1962; see for review Anderson 1970; Corbet 1978). Thus hybridization between sympatrically occuring closely related *Martes* species is not only possible, but also perhaps even likely.

Two molecular markers have been used so far in evaluating the genetic relationship between *M. melampus* and *M. zibellina*, firstly the restriction fragment length polymorphism (RFLP) of the nuclear ribosomal DNA (rDNA) spacer (Hosoda et al. 1993, 1997), and secondly the mitochondrial cytochrome *b* (cyt *b*) gene sequences (Masuda and Yoshida 1994b). We used these markers to reveal the extent of intra- and inter-specific variation between the two species. We also examined one *M. melampus* from Hokkaido genetically,

and discussed the possibility of interspecies hybridization in nature.

Materials and methods

DNA samples

DNA was extracted from liver tissues of *M. zibellina* and *M. melampus* from a range of localities, using the method described by Maniatis et al. (1982) (see Table 1 and Fig. 1).

Direct sequencing of cyt b gene

Semi-nested polymerase chain reactions (PCRs), and direct sequencing were performed following methods previously described by Suzuki et al. (1997). The universal primers L14724 and H15915 (Kocher et al. 1989) were used for the first PCR, and R-L14724 and U-H15155 (Suzuki et al. 1997) were used for the second PCR. Then both strands of the second PCR product were sequenced directly by an automated sequencer (model 373A; ABI).

Table 1. Profiles of samples used and specific types of mtDNA.

Genus	Species	Serial no. and locality	Sample no.	Sex	Type of mtDNA
Martes					
	M. zibellina				
		1. Khabarovsk, Russia	VK183	unknown	Mzi1
			HS949	unknown	Mzi2
		2. Hokkaido, Japan	TH043	male	Mzi3
			TH044	male	Mzi3
			TH045	male	Mzi3
			TH047	male	Mzi3
			TH053	male	Mzi3
			TH107	male	Mzi3
			HEG293	male	Mzi3
	M. melampus				
		3. Hokkaido, Japan	TH048	male	Mme1
		4. Tochigi, Japan	TH017	female	Mme1
		5. Niigata, Japan	TH006	male	Mme1
		6. Wakayama, Japan	TH020	male	Mme1
		7. Shimane, Japan	TH018	male	Mme1
		8. Miyazaki, Japan	HS862	male	Mme1
		9. Tokushima, Japan	TH131	male	Mme1
			TH176	male	Mme1
			TH010	male	Mme2
			TH175	female	Mme2
		10. Tsushima Is., Japan	TH004	female	Mme3
			TH005	male	Mme3
			TH007	male	Mme3
Mustela					
	M. itatsi				
		11. Aomori, Japan	TH089	male	Mit
	M. sibirica				
		12. Vladivostok, Russia	HS1223	male	Msi

Fig. 1. Localities from which individuals of *Martes zibellina* (closed circles) and *M. melampus* (open circles) were collected for this study. (Numbers beside collection points refer to Table 1).

Construction of phylogenetic trees

We produced a matrix of sequence divergence for all possible combinations of mtDNA sequences (Table 1) using the computer program DNADIST in PHYLIP 3.5 (Felsenstein 1993) and Kimura's two parameter method (Kimura 1980). We constructed a phylogenetic tree using the neighbor-joining (NJ) method (Saitou and Nei 1987) using the computer program NEIGHBOR in PHYLIP 3.5. Confidence levels for each grouping were calculated using the bootstrap program SEQBOOT (with 1,000 replications) in the PHYLIP package. The tree was produced using the CONSENCE program in the PHYLIP package.

Analysis of rDNA RFLP

Variations in rDNA were examined using Southern's blot analysis (Southern 1975). For the construction of restriction maps of the various types of rDNA repeating units, the genomic DNAs were digested with twelve restriction enzymes: *Aat*I, *Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sac*I and *Xba*I. The digested DNAs blotted on nylon filters were hybridized sequentially with three ^{32}P-labeled rDNA probes as described by Hosoda et al. (1993).

Results

Variations in mtDNA

We determined partial sequences (402 base pairs (bp)) of cyt *b* genes of 22 martens, and two weasels (*Mustela sibirica* and *M. itatsi*) as an outgroup (Table 1, Fig. 2), and calculated the sequence divergence. Based on the extent of the sequence divergence, we constructed a phylogenetic tree using the NJ method (Fig. 3). The sequence divergence between *Martes melampus* and *M. zibellina* was 2.6–3.9% (3.3% on average), which was similar to the degree of divergence found between *M. sibirica* (Msi) and *M. itatsi* (Mit), 4.3%. Both *M. melampus* (Mme) and *M. zibellina* (Mzi) had three haplotypes (Mme1, Mme2, Mme3 and Mzi1, Mzi2, Mzi3). *Martes melampus* of Honshu (Niigata, Tochigi, Shimane and Wakayama prefectures) and Kyushu (Miyazaki) were monomorphic (Mme1 only), while the Shikoku population had two haplotypes (Mme1 and Mme2), and two individuals from Tsushima

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               10         20         30         40         50         60         70         80
Mzi1   ATGACCAACA TTCGTAAAAC TCACCCACTA GCTAAAATCA TCAACAATTC ATTCATCGAC TTACCTGCCC CATCAAACAT
Mzi2   .......... .......... .......... ..C....... .......... .......... ........T. ..........
Mzi3   .......... .......... .......... ..C....... .......... .......... ........T. ..........
Mme1   .......... .......... .......... .......... .......... .......... ........T. ..........
Mme2   .......... ....C..... .......... .......... .......... .......... ........T. ..........
Mme3   .......... .......... .......... .......... .......... .......... ........T. ..........
Mit    .......... ....C..... C......... A.C....... ........C. ......T..T .....C.... ..........
Msi    .......... ....C..... C......T.. A.C.....T. .......C.. ....G.A... C....C.... ..........

               90        100        110        120        130        140        150        160
Mzi1   TTCCGCATGA TGAAATTTCG GCTCCCTCCT TGGAATCTGT CTGATCCTAC AGATTCTTAC AGGTTTATTT CTAGCCATAC
Mzi2   .......... .......... .......... .......... .......... .......... .......... ..........
Mzi3   .......... .......... .......... .......... .......... .......... .......... ..........
Mme1   .......... ....C..... .......... .......C .. ..A...... .......... .......... ..........
Mme2   .......... ....C..... .......... .......C .. ..A...... .......... .......... ..........
Mme3   .......... ....C..... .......... .......C .. ..A...... .......... .......... ..........
Mit    ...A..G... ....C..... .......T.. C........ ..C ..A..TA.T. .......... .......... T....T....
Msi    ...A...... .......... .......T.. C........ ..C ..A..TA.T. .......... .......... T.........

              170        180        190        200        210        220        230        240
Mzi1   ACTACACATC AGACACAGCC ACAGCCTTCT CATCAGTCAC CCACATTTGC CGAGACGTCA ACTACGGCTG GATTATCCGA
Mzi2   .......... .......... .......... .......... .......... .......... .......... ......T...
Mzi3   .......... .......... .......... ....G..... .......... .......... .......... ......T...
Mme1   ....T..... .......... .......... .......... .......... .......... ....A.. A........
Mme2   ....T..... .......... .......... .......... .......... .......... ....A.. A........
Mme3   ....T..... .......... .......... .......... .......... .......... ....A... A........
Mit    ....T..... .......... .......... ........T. .......... ...T..C..T .......... ....T..... A..C......
Msi    ....T..... .......... .......... ........T. .......... ...T.....T .......... ....T..... A..C......

              250        260        270        280        290        300        310        320
Mzi1   TATATACATG CCAACGGGGC TTCCATATTC TTCATCTGCC TGTTCCTGCA CGTCGGACGG GGTTTATACT ATGGATCTTA
Mzi2   .......... .......... .......... .......... .......... .......... .......... ..........
Mzi3   .......... .......... .......... .......... .......... .......... .......... ..........
Mme1   ..C....... .......... .......... .......... .......... .......... ..CC...... ..........
Mme2   ..C....... .......... .......... .......... .......... .......... ..CC...... ..........
Mme3   ..C....... .......... .......... .......... .......... .......... ..CC...... ..........
Mit    .......... .A.....A.. C......... ..T....... .......A.. ...A..G..A ........T. .C........
Msi    ..C.....C. .A.....A.. C......... .......... .......A.. ...A..G..A ........T. .C........

              330        340        350        360        370        380        390        400
Mzi1   TATATACCCC GAAACATGGA ACATTGGTAT CATCCTATTA TTCGCAGTTA TAGCAACAGC ATTCATAGGT TACGTTCTGC CA
Mzi2   .......... .......... .......... .......... ........C. .......... .......... .......... ..
Mzi3   .......... .......... .......... .......... ........C. .......... .......... .......... ..
Mme1   .......... .......... .......C.. .......... .......... .......... .......... .......... ..
Mme2   .......... .......... .......C.. .......... .......... .......... .......... .......... ..
Mme3   .......... .......... .......C.. .......... .......... .......... .......... .......... ..
Mit    .....T.A.. ........A. ....C..C.. T...T..... ........C. .......T.. .......... ......T.A. ..
Msi    .....TAA.. ........A. ....C..A.. T...T....G ........C. .......T.. .......... ......T.A. .G
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Fig. 2. Partial sequences of the mitochondrial cytochrome *b* gene for martens and the outgroup, *Mustela*. The abbreviations of the gene types are as in Table 1.
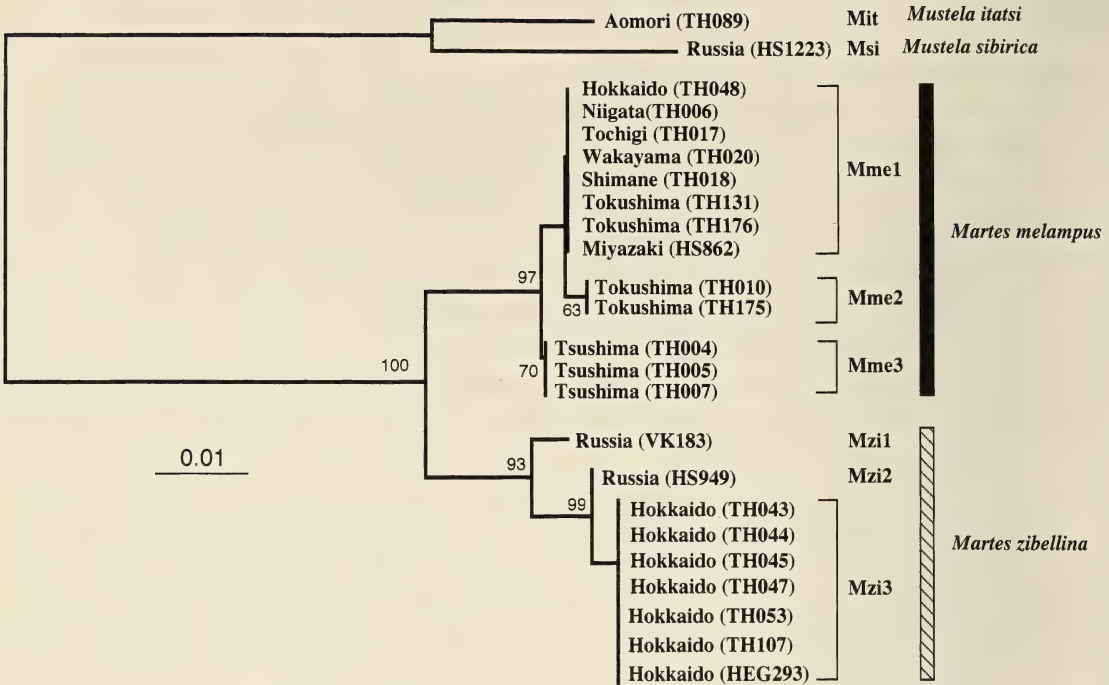
Fig. 3. A neighbor-joining tree constructed from the cytochrome *b* gene sequences (402 bp) of 22 individuals of *Martes*. Two species of *Mustela* were used as an outgroup. The bootstrap scores above each branch are expressed in percentages of 1,000 replicates.

possessed one specific type, Mme3. All seven animals of *M. zibellina* from Hokkaido shared a common sequence (Mzi3), while two individuals from Khabarovsk had different types (Mzi1 and Mzi2) revealing a rather considerable extent of intra-population variation (1.0% sequence divergence). There was, however, only 0.2% (1/402 bp) sequence divergence between one of the continental types, Mzi2, and the Hokkaido type, Mzi3.

Variation in rDNA

We examined the nuclear rDNA-RFLP with 12 restriction enzymes and compared 24 restriction sites along with the external spacer regions of rDNA from 14 *M. melampus* and *M. zibellina* (Table 1). As Hosoda et al. (1993) reported, only the *Bgl*II site(s) located upstream from the 5' end of the 18S rRNA gene differs between the two species: 19 kb for *M. melampus* and 15 kb for *M. zibellina*. We found no variation within the species.

Martes melampus in Hokkaido

Since one yellow animal, obtained from Sapporo, was morphologically similar to *M. melampus*, we examined genetic characteristics of the two molecular markers. It proved to have a cyt *b* sequence Mme1 specific to *M. melampus* (Table 1). With the 18S rDNA probe, the 19 kb *Bgl*II band was detected, but the 15 kb band, which is specific to *M. zibellina*, was definitely absent. Since patterns of nuclear rDNA may reflect a specific kind of genomic status (i.e., the band represents a hundred copies of repeating units that are generally dis-

persed among different chromosome loci), the rDNA profile is likely to indicate the status of most of the other nuclear genes in this individual.

Discussion

Interspecies relationships

One of our aims in this study was to evaluate the genetic relationship between *M. melampus* and *M. zibellina* in Hokkaido, from both evolutionary and current perspectives. The extent of the divergence of the cyt *b* gene (3.3%) between these two species was almost the same as the divergence between the Japanese and Siberian weasels (Fig. 3; Masuda and Yoshida 1994a). Masuda and Yoshida (1994a) estimated, on the basis of the sequence divergence of the cyt *b* gene region (375 bp) is 4.0–4.3%, that the split between the two weasel species occurred some 1.6–1.7 million years ago. The differentiation of the two marten species is also evident from the rDNA-RFLP data (Hosoda et al. 1993), leading us to presume that these two species have also genetically differentiated considerably, during the last one or two million years. Although the existence of land bridges between Honshu and Hokkaido during recent ice ages may have presented several opportunities for hybridization, it is also possible that the two species could have maintained their own genetic independence without exchanging genetic elements.

In order to evaluate the possibility of genetic hybridization between the two species under natural condition, it was necessary to examine individuals from a site where both species occur. Considering the limited literature available (Inukai 1975) and the patterns of mtDNA and rDNA, it seemed most likely that *M. melampus* from Sapporo, in western Hokkaido, would be the descendants of translocated martens from Honshu which were then released or escaped from fur farms half a century ago. We did not find any sign of interspecific hybridization in this sample, or among seven sable from Teshio (northern Hokkaido), indicating that hybridization between these two species is unlikely. As there have been, however, several cases of natural hybridization between continental martens, such as between *M. zibellina* and *M. martes* (Anderson 1970; Corbet 1978), a continuous survey of a suitable sample size is necessary to finally conclude the possibility of natural hybridization between *M. melampus* and *M. zibellina*. Such a study would also be useful for estimating the risk of genetic contamination through introduced animals.

Geographic variation

Mitochondrial DNA sequence variation within each species was not great (Fig. 3). *Martes melampus tsuensis* from Tsushima are distinguishable as a separate subspecies from other geographic populations on the basis of their morphological differences (see Introduction, Anderson 1970). The types of mtDNA differed only slightly between the subspecies, with only one base substitution among the 402 bp sequence. This result suggests that there has been some recent (in geological terms) genetic exchange between the Tsushima and mainland Japanese populations of this species, probably during the late Pleistocene.

We examined *M. zibellina* from just two localities, Teshio, in Hokkaido (seven individuals), and Khabarovsk, Russia (two individuals); and found no substantial differences in the cyt *b* of the two populations. The Hokkaido type Mzi3 differed from the Russian type Mzi2 by only one base among the 402 sites (Fig. 2), indicating that there has been recent

divergence between Hokkaido and the Russian Far East populations.   In the case of the red fox, *Vulpes vulpes*, the mtDNA D-loop sequences of individuals from Hokkaido and from the Russian Far East are involved in a clade with a small extent of polymorphism (Tsuda et al. 1997).   These results, for both fox and sable, are consistent with the fact that Hokkaido was periodically connected to the continent by ice age land bridges, and only finally isolated within the last 10,000 years (e.g. Oshima 1990).   It is now evident that carnivorous mammals ranged across both the Russian Far East and Hokkaido exchanging genetic elements between these now isolated geographic regions, during a geologically recent period.

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A karyological analysis of the Korean red-backed vole, *Eothenomys regulus* (Rodentia, Muridae), using differential staining methods

Masahiro A. Iwasa[1], Sang Hoon Han[2] and Hitoshi Suzuki[3]

[1,3]*Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*
[2]*Ecosystem Conservation Division, Ministry of Environment, 1, Joongang-dong, Kwacheon, Kyunggi-do 427-760, Korea*

Abstract. The conventional and G- and Q-banded karyotypes of the Korean red-backed vole *Eothenomys regulus* (2n=56) are described here for the first time. The autosomes were found to be composed of 26 pairs of acrocentrics and one pair of metacentrics, as in other species of red-backed voles. Side-by-side pair-matching analysis revealed that the G-banding patterns of *E. regulus* were essentially identical to those of the grey red-backed vole *Clethrionomys rufocanus*, and therefore the karyotype of *E. regulus* was of a "*rufocanus*" type, not of a "*glareolus*" type, which is characterized by 1–9 translocation. The sex chromosomes of *E. regulus* were found to be composed of a large subtelocentric X chromosome and a medium-sized subtelocentric Y chromosome, closely resembling those of *E. smithii* in both size and morphology. Both X and Y sex chromosomes were indistinguishable between these species, as far as conventional staining is concerned. Further analysis indicated, however, that *E. regulus'* Y chromosome has a large C-band area on the terminal half of its long arm, whereas *E. smithii* has a large C-band area on the proximal half of its long arm. Such C-band patterning implies the involvement of the Y chromosome in paracentric inversion during the course of speciation.

Key words: karyotype, *Eothenomys regulus*, the Korean red-backed vole, sex chromosomes.

The Korean red-backed vole was first described as *Craseomys regulus* by Thomas (1907), on the basis of the type specimen collected at Min-gyong, Korea. Now, this species is widely regarded as belonging to the genus *Eothenomys* (Corbet 1978; Kaneko 1990; Corbet and Hill 1991; Musser and Carleton 1993), although on the basis of molecular data from mitochondrial, and nuclear ribosomal DNA, its inclusion in the genus *Clethrionomys* has also been proposed (Wakana et al. 1996; Suzuki et al. 1999). Thus the taxonomic status of this vole remains uncertain. With the exception of the Korean species, the karyotypes of all of the East Asian red-backed vole species have been studied. Differential staining methods have shown that all of them share the same diploid number 2n=56 with essentially 26 pairs of

acrocentrics (or subtelocentrics) and one pair of metacentrics, showing a high degree of karyotypic similarity (Tsuchiya 1981; Ando et al. 1988; Kashiwabara and Onoyama 1988; Yoshida et al. 1989; Sokolov et al. 1990; Obara et al. 1995; Kitahara and Harada 1996). Detailed G-banding analysis has revealed, however, that the red-backed vole species complex can be divided, karyologically, into two groups, the "*glareolus*" and the "*rufocanus*" groups (Gamperl 1982; Iwasa 1998). The "*glareolus*" group is characterized by the 1-9 translocation which can be seen in *C. glareolus*, *C. rutilus*, *C. gapperi* and *C. californicus* (Modi 1987; Modi and Gamperl 1989; Obara et al. 1995), whereas the "*rufocanus*" group (*C. rufocanus*, *C. rex* (dealt with as a synonym of *C. montanus*) and two Japanese species *E. andersoni* and *E. smithii*) shows no such translocation (Obara 1986; Ando et al. 1988; Kashiwabara and Onoyama 1988; Yoshida et al. 1989; Sokolov et al. 1990; Obara et al. 1995; Kitahara and Harada 1996).

The purpose of this study was to make the first examination of the karyotype of *E. regulus*, and to compare it with those of related species, so as to be able to ascertain, from a cytogenetic perspective, the phylogenetic position of *E. regulus* among the East Asian red-backed vole species.

Materials and methods

Two male *Eothenomys regulus* were captured, using Sherman live-traps, at Tonmyon-ri, Sesanmyon, Ponghwa-gun, Kyongsangbuk-do, Korea. They were identified on the basis of their cranial and dental characteristics as described by Kaneko (1990) (see Table 1 and Fig. 1), and preserved in 70% ethanol as specimens HEG22-97 and HEG49-97. Six
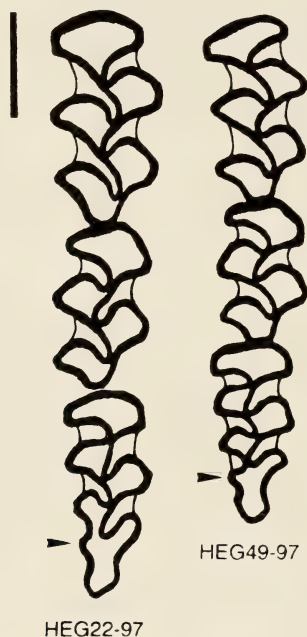

HEG49-97

HEG22-97

Fig. 1. Enamel patterns of the right upper molars of the *Eothenomys regulus* specimens examined in this study. (Arrowheads indicate the fourth outer small salient angle. Bar = 1 mm. See Table 1 for specimen number).

Table 1. Morphological measurements of the Korean red-backed vole, *Eothenomys regulus*, examined in this study.

Specimen No.	Sex	Capturing date	T.L. (mm)	T. (mm)	T.R. (%)	H.F. (mm)
HEG22-97	male	25 Apr. 1997	142.5	36.0	33.8	19.3
HEG49-97	male	24 Apr. 1997	141.0	40.0	39.6	18.2

T.L.: Total length; T.: Tail length; T.R.: Tail rate; H.F.: Hind foot length.

Table 2. Number of cells observed.

Specimen No.	Conv. Giemsa	G-band	Q-band	C-band
HEG22-97	30	15	28	16
HEG49-97	62	36	115	22
total	92	51	143	38

Clethrionomys rufocanus collected in Hokkaido, and six *E. smithii* collected in Shikoku, were used for a comparison of the sex chromosomes.

Chromosome preparations were made from bone marrow cells after short-term culture (40 min at 37°C) in MEM containing 15% fetal calf serum and colchicine (final concentration 0.025 g/ml). The bone marrow cells were treated in 0.075 M KCl at 37°C for 18 min, followed by fixation with Carnoy's fixative (methanol: acetic acid = 3:1). Cell suspensions were dropped on slides and air-dried. Chromosomes were analyzed by both conventional and differential staining methods. For the latter staining method C-, Q- and G-bands were examined following methods described by Caspersson et al. (1971), Sumner et al. (1971) and Sumner (1972; see Table 2).

Results and discussion

Two red-backed vole specimens collected from the Korean Peninsula were examined intensively in order to determine their specific identification on the basis of their morphological features since two very similar species of voles, *E. regulus* and *C. rufocanus*, have been reported from the region (Corbet 1978). The two species closely resemble each other in morphology, but *E. regulus* has a specific "complex form" of enamel patterning on the upper third molar (Kaneko 1990), which is distinguishable from that of *C. rufocanus*. Our two specimens both had "complex form" upper third molars (Fig. 1), and so were confirmed as *E. regulus* (Kaneko 1990).

Eothenomys regulus was confirmed as having 26 pairs of acrocentrics and one pair of metacentrics, which was the smallest pair in the complement (Fig. 2a). The autosomes and the X chromosome (excluding its short arm) had G-banding patterns identical with those of *C. rufocanus* (Fig. 3) and other Japanese red-backed vole species. Thus, karyologically, *E. regulus* belongs to the "*rufocanus*" group, which has no 1–9 translocations (Gamperl 1982; Obara et al. 1995). As expected, the Q-banding patterns (Fig. 2b) were almost identical to the G-banding patterns (Fig. 3); the bright bands basically corresponded to G-positive bands. Centromeric regions showed dull fluorescence in all chromosomes after Q-banding.

In contrast to the highly consistent constitution of the autosomes of red-backed vole species, the sex chromosomes showed both inter- and intraspecific variation. The sex chro-
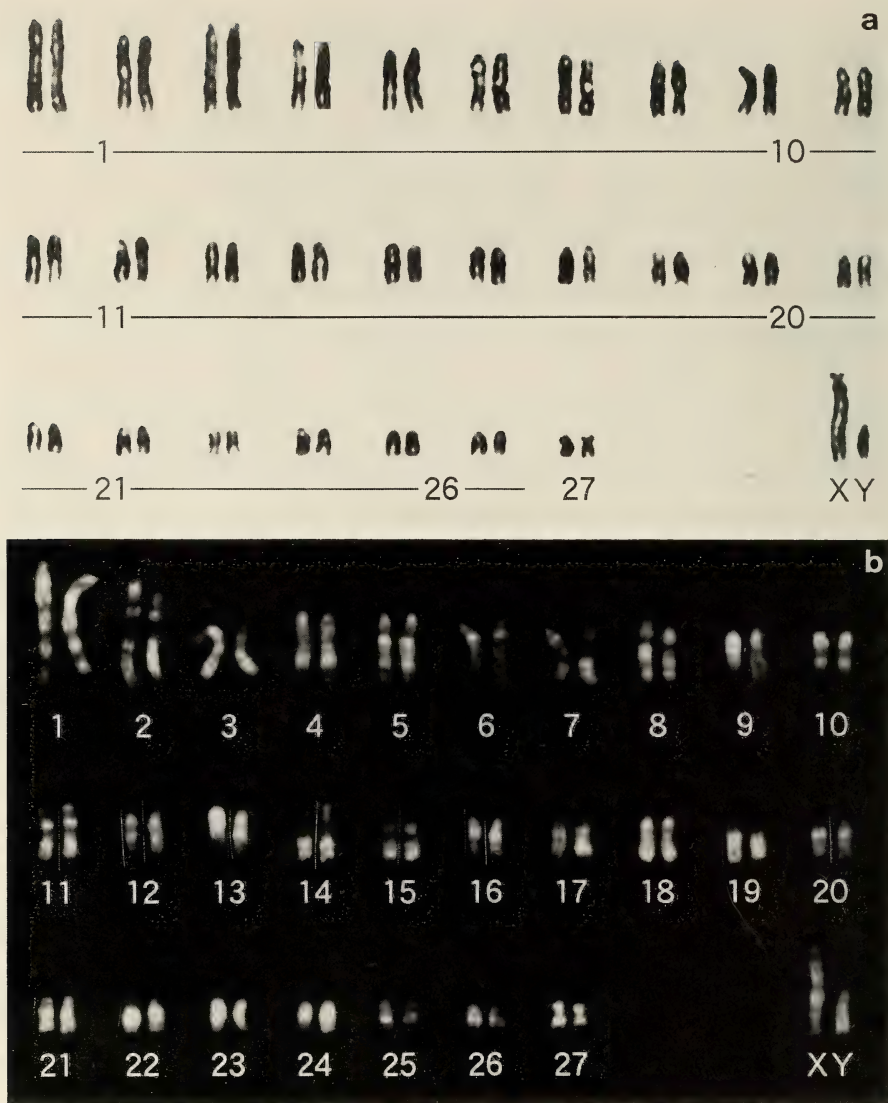
Fig. 2.   Conventionally stained (a) and Q-banded (b) karyotypes of *Eothenomys regulus*.

mosomes of *E. regulus* proved to be composed of a large subtelocentric X and a medium-sized subtelocentric Y chromosome.   Such a combination is markedly different from the XY chromosomes of *C. rufocanus*, which has a large acrocentric X and a small acrocentric Y chromosome (Fig. 4).   Two distinct karyological forms of *E. smithii* have been reported, one with a small Y chromosome (the so-called *smithii* form of south-western Honshu and Shikoku (Fig. 4; Ando et al. 1988), and the other with a large Y chromosome the *kageus* form of central Honshu (Ando et al. 1988).   The Y chromosome of *E. regulus* was equivalent in length to that of the small *smithii* form of *E. smithii*.   A detailed comparison of the C-bands of *E. regulus* and *E. smithii* indicated, however, the possibility of a structural rear-
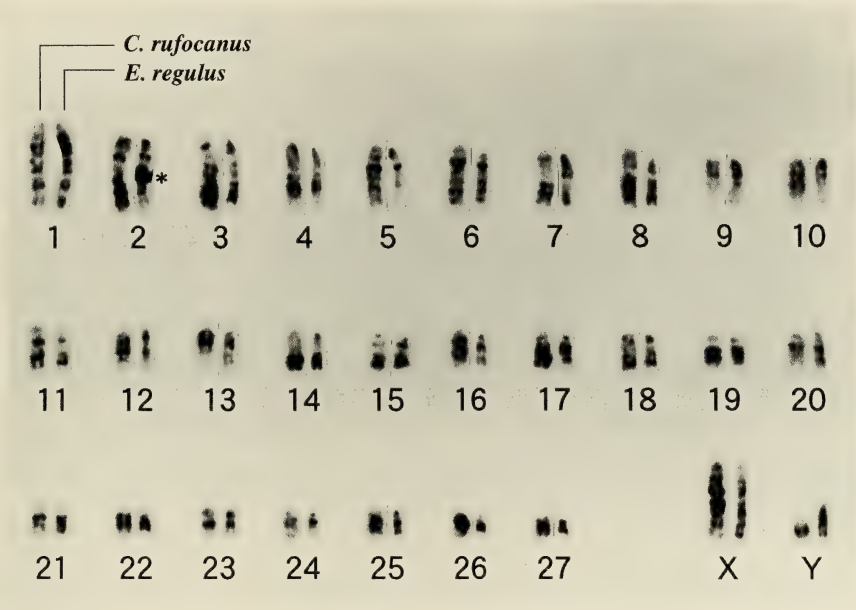
Fig. 3. Composite karyotype of *Clethrionomys rufocanus* and *Eothenomys regulus* prepared by side-by-side arrangement on the basis of G-band homology. (Left = *C. rufocanus*. Right = *E. regulus*. The asterisk indicates overlapping chromosomes).

rangement of the Y chromosome. The Y chromosome of *E. regulus* has a large C-band area on the terminal half of its long arm, whereas that of *E. smithii* has a similarly sized C-band on the proximal half of its long arm (Fig. 5). Similar C-band patterns in *E. smithii* have also been described by Ando et al. (1988) and Yoshida et al. (1989). Such interspecific differences in C-banding patterns can be explained by the occurrence of paracentric inversion involving most of the long arm of the Y chromosome during the course of speciation (Fig. 5).

The Y chromosome morphology suggests a closer relationship between *E. regulus* and *E. smithii* than with *C. rufocanus*, in which the Y chromosome is small and metacentric in the Primorskyi region of Russia, and small and acrocentric in Hokkaido, Japan (Vorontsov et
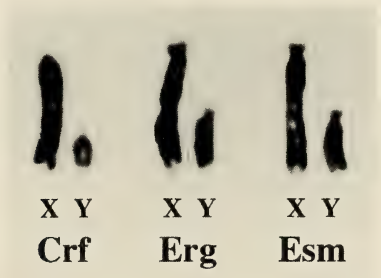

Fig. 4. Conventionally stained X and Y chromosomes of *Clethrionomys rufocanus* (Crf), *Eothenomys regulus* (Erg) and *E. smithii* (Esm).
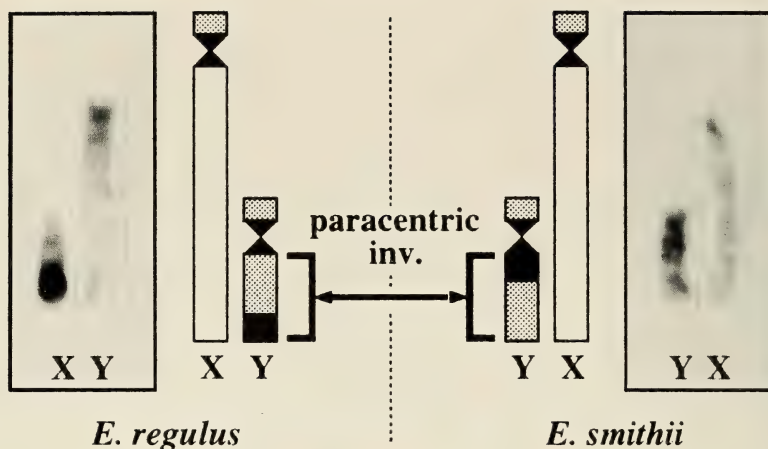
Fig. 5. C-banding patterns in the X and Y chromosomes of *Eothenomys regulus* and *E. smithii* shown by photographs and ideograms.

al. 1980; Tsuchiya 1981; Obara 1986; Yoshida et al. 1989). This chromosomal evidence is consistent with the fact that adult *E. regulus* and *E. smithii* both have rootless molars. In contrast, however, molecular phylogenetic data, on the variation of nuclear ribosomal and mitochondrial DNA, suggests a closer relationship between *E. regulus* and *C. rufocanus*, than with *E. smithii* (Wakana et al. 1996; Suzuki et al. 1999). The Shikoku *E. smithii* population, however, has specific mitochondrial sequences that differ from those of the Honshu and Kyushu populations, but which show affinities with those of both *C. rufocanus* and *E. regulus* (Suzuki et al. 1999). Thus, the phylogenetic relationships of these three red-backed voles, *E. regulus*, *E. smithii* and *C. rufocanus* are extremely complicated and as yet unresolved. Our cytogenetic and molecular findings indicate that interspecific genetic exchange may have played at least a partial role in complicating the genetic constitution of these three red-backed vole species. An analysis of the sequence variations in the genes specific to the X and Y chromosomes, and molecular cytogenetic analysis of the Y chromosomal C-heterochromatin, is considered likely to yield valuable information towards a more precise understanding of the phylogenetic relationships of these species. We are currently in the process of examining the relationships between the red-backed voles from these standpoints.

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The genetic status of two insular populations of the endemic spiny rat *Tokudaia osimensis* (Rodentia, Muridae) of the Ryukyu Islands, Japan

Hitoshi Suzuki[1], Masahiro A. Iwasa[2], Nobuo Ishii[3], Hiroko Nagaoka[4] and Kimiyuki Tsuchiya[5]

[1,2]*Graduate School of Environmental Earth Science, Hokkaido University, North 10, West 5, Kita-ku, Sapporo 060-0810, Japan*
[3,4]*Japan Wildlife Research Center, Yushima 2-29-3, Bunkyo-ku, Tokyo 113-0034, Japan*
[5]*Experimental Animal Center, Miyazaki Medical College, 5200 Kihara, Kiyotake-cho, Miyazaki 889-1692, Japan*

Abstract.   We examined the geographic variation of *Tokudaia osimensis* through the analysis of mitochondrial cytochrome *b* (cyt *b*) gene sequences and the restriction fragment length polymorphism (RFLP) in the nuclear ribosomal RNA gene (rDNA), using samples collected from Tokuno-shima and Amami-oshima in the Ryukyu Islands.   The two populations show intrinsic karyological variation (Tokuno-shima, 2n=45; Amami-oshima, 2n=25). Sequences of the cyt *b* gene differed considerably between the two island populations.   The extent of the sequence divergence among 1,140 bp of the gene was calculated to be 0.088 using the Kimura two parameter method, and was comparable to those between related species of rodents such as within genus *Mus* or *Rattus*.   The extent of the differentiation in the rDNA-RFLP was also high.   Three out of 22 restriction site variants were found to be fixed in the nuclear rDNA arrays of hundreds of copies in either one of the two island populations.   These intensive inter-populational differences indicate that the two island populations may have been isolated for a considerable period of evolutionary time, probably several millions of years, despite there having been several opportunities for renewed genetic contact during the Pleistocene ice ages.   Our data strongly suggest that the current taxonomic status of the populations of the two islands, Amami-oshima and Tokuno-shima, which regards them conspecific, should be reviewed.

Key words: geographic variation, cytochrome *b* gene, nuclear rDNA-RFLP, Ryukyu Islands, *Tokudaia osimensis*.

The Nansei Shoto or Ryukyu Islands, Japan's southernmost islands, harbor a unique fauna and flora, and the central region, consisting of the three islands of Amami-oshima, Tokuno-shima and Okinawa and adjacent islets, is especially rich and a center of endemism. Three genera of the following mammals are endemic to this small area of islands: the Ryukyu spiny rat *Tokudaia osimensis*; the Ryukyu long-haired rat *Diplothrix legata*; and the Amami

[1]*E-mail: htsuzuki@ees.hokudai.ac.jp*

rabbit *Pentalagus furnessi* (Corbet and Hill 1991; Musser and Carleton 1993; Hoffmann 1993; Abe 1994; Kaneko and Murakami 1996). Because of their uniqueness and zoological importance, all three are protected as natural monuments by the Japanese government and also regarded as endangered species (IUCN 1996; Kawamichi 1997). These three species are also considered to be symbolic of the significant biodiversity of the Central Ryukyus, and for the conservation of the fauna of the islands. Moreover, their distribution and status provides invaluable information towards an understanding of the historical episodes of the Ryukyu fauna as well as contributing to an understanding of general evolutionary issues.

Tokudaia osimensis, in particular, raises various interesting scientific issues. The species shows intrinsic karyological features in its autosomes and sex chromosomes, with the diploid numbers of both females and males being 44 in Okinawa, 45 in Tokuno-shima, and 25 in Amami-oshima (Honda et al. 1977, 1978; Tsuchiya et al. 1989). Since the Y chromosome in the Tokuno-shima and Amami-oshima island populations disappears (Honda et al. 1977, 1978), and the animals from Amami-oshima are shown to lack the Sry gene (Soullier et al. 1998), an unusual sex-determining system must have been evoked in these populations (Honda et al. 1977; Tsuchiya et al. 1989; Soullier et al. 1998; Xiao et al. 1998). However, neither the evolutionary process leading to these differences, nor the biological implications of the differences have been elucidated.

The evolutionary history of the populations of *T. osimensis* and the origin of this lineage have long been debated (for review see Kawamura 1989). A recent molecular phylogenetic study (Suzuki et al. 1999b) has revealed that *T. osimensis*' lineage is distinct from the other members of the subfamily Murinae examined so far, including *Apodemus*, which had been considered a likely candidate for the sister lineage of *Tokudaia* based on molar morphology (Kawamura 1989). An assessment of the genetic diversity within this species is inevitably needed for back ground information to elucidate the above biological problems, and to resolve the unsettled taxonomic status of the three island populations of *T. osimensis*. Although recently each of the three populations is presumed to be a distinct species (Honda et al. 1977; Tsuchiya et al. 1989; Musser and Carleton 1993), there has only been limited research on this species with such complicated genetic property using molecular markers (Tsuchiya et al. 1989).

In a preliminary study using a limited number of restriction enzymes we found substantial genetic differences between populations of *T. osimensis* from Tokuno-shima and Amami-oshima, based on restriction fragment length polymorphisms (RFLP) of mitochondrial DNA (mtDNA) and nuclear ribosomal RNA genes (rDNA) (Tsuchiya et al. 1989). This study was conducted therefore to improve our knowledge of the molecular phylogeny of the Tokuno-shima and Amami-oshima island populations, by examining a whole sequence for the mitochondrial cytochrome *b* (cyt *b*) gene and the rDNA-RFLP with more additional restriction enzymes.

Materials and methods

Animals

We have tentatively followed Abe's (1994) classification and accepted that *Tokudaia* consists of just one species (*T. osimensis*) with three island populations. Five individuals of *T. osimensis* were examined, two from Tokuno-shima and three from Amami-oshima. With

the exception of one sample from Amami-oshima, the samples were the same as those previously used by Tsuchiya et al. (1989). The new sample (sample no. HS1142) from Amami-oshima was collected at Tatsugo, 28 February 1996 through a wildlife survey conducted by Japan Wildlife Research Center (Environment Agency of Japan 1995).

Sequencing and phylogenetic analysis

Nuclear DNA extraction, Southern blot analysis and the construction of restriction maps for the rDNA repeating unit type (repetype), were all carried out following Suzuki et al's (1994a) methodology. The cyt *b* region was analyzed using nested polymerase chain reactions and a direct sequencing method as described previously by Suzuki et al. (1997, 1999b).

In order to estimate the sequence divergence from restriction site variation among rDNA repetypes, we compared the arrangement of the restriction sites between the pairs of repetypes and then counted the common and divergent sites (Suzuki et al. 1994a). To do this, we used Gotoh et al's (1979) method, in which backward mutations and parallel mutations are taken into account, to produce a matrix of sequence divergence among all possible combinations of repetypes.

To estimate the sequence divergences from sequences of the cyt *b* gene, we used the two parameter method (Kimura 1980) and MEGA (Kumar et al. 1993).

Results

Cyt b sequences

Fragments of the cyt *b* gene, from each of the five specimens, consisting of 402 bp were determined, and it was found that each island population had unique sequences. We then sequenced the entire gene region of the cyt *b* gene of one individual from Tokuno-shima (the nucleotide sequence can be reached in the DDBJ, EMBL and GenBank with following accession number: AB029429) and compared it with that of the previously described sample from Amami-oshima (Suzuki et al. 1999b), calculating sequence divergences (see Kimura 1980) taking into consideration complete substitution (d), and only transversional substitution (dv; see Table 1). The extent of transversional substitution amounted to 0.026, which is comparable to that between species of *Rattus-Diplothrix* and *Mus* ($dv = 0.014$–0.016; Table 1). The extent of complete substitution ($d = 0.088$) was also extremely high when compared with other cases of intraspecific sequence divergences within mammalian species (Avise et al. 1998; Johns and Avise 1998; Table 1 for the case of *Glirulus japonicus*) and rather comparable to those among congeneric mammalian species (Johns and Avise 1998). Such high degrees of divergence in the cyt *b* sequences were congruent with our previous study with the mtDNA-RFLP (Tsuchiya et al. 1989).

rDNA-RFLP

We carried out Southern blot analysis with the two island samples using 12 restriction enzymes. Among the enzymes examined the *Kpn*I bands, with both the 18S and 28S probes, remained at higher molecular weight position without any indication of digestion with this enzyme in samples from both islands. Thus, we considered there to be no *Kpn*I site located in the spacer region in these populations. Restriction maps were constructed taking into consideration the banding patterns (Fig. 1). Interestingly, the Amami-oshima sample's

Table 1. Comparison of sequence divergences between related species and among geographic populations in small mammals. Sequence divergences in the cytochrome *b* gene (1,140 bp) were calculated using Kimura's (1980) two parameter method considering all substitutions at all codon positions (*d*) and transversions at all codon positions (*d*v).

Taxa compared	Substitution considered	
	d	*d*v
Between geographic populations		
1. *Tokudaia osimensis*		
'Amami-oshima' vs 'Tokuno-shima'	0.088	0.026
2. *Mus musculus**		
M. m. domesticus vs *M. m. musculus*	0.024	0.004
3. *Glirulus japonicus***		
'Wakayama' vs 'Yamanashi'	0.075	0.012
Between species within *Mus* and *Rattus**		
4. *M. musculus* vs *M. spretus*	0.091	0.014
5. *R. rattus* vs *Diplothrix legata*	0.102	0.016
Between genera *Mus* and *Rattus**		
6. *M. musculus* vs *R. norvegicus*	0.186	0.082

  * Suzuki et al. (1999b). The genus *Diplothrix* is a member of a *Rattus* group in the molecular phylogenetic view.

** Suzuki et al. (1997; unpublished data)

repeating type was heterogeneous within a genome as depicted in the *Hind*III and *Xba*I sites upstream of the 18S rRNA gene and *Eco*RI, and *Dra*I sites downstream of the 28S rRNA gene (Fig. 1). In contrast, the banding patterns of the Tokuno-shima specimens were monotypic within a genome at each restriction site. These phenomena can be explained either by there being a large population on Amami-oshima or by some prevention of the homogenization process (including DNA recombination) within a genome in the Amami-oshima population. The former postulates that the banding patterns of individuals from a large population size tend to show polymorphic state rather than those from a small population size (Suzuki et al. 1994b). Although there is no substantial data on population size of this species, the area of Amami-oshima is about three times as large as that of Tokuno-shima. In the latter case, if rDNA clusters coexist onto terminal and interstitial regions of chromosomes, recombination between non-homologous chromosomes would be unfavorable since it may cause abnormal chromosomal changes with serious damage to the cell. We just presume a possibility that a rDNA cluster(s), which often locate distal portions of chromosomes accompanied by heterochromatic regions but not euchromatic one, as in the cases of *Mus* and *Rattus* (Babu and Verma 1985), incorporated into inside chromosomes by chromosomal rearrangement in the Amami-oshima population (2n=25).

The difference between the geographic populations became more conspicuous during examination of the rDNA-RFLP. According to Gotoh et al's (1979) method, inter-populational sequence divergence was calculated to be approximately 2.3%. This extent was considered likely to be as high as between distantly related local populations such as of the Japanese dormouse *Glirulus japonicus* (2.9–3.3%, Suzuki et al. 1997), and between closely related species of red-backed voles (genera *Clethrionomys* and *Eothenomys*) in Japan (1.9–2.3%, Wakana et al. 1996; Suzuki et al. 1999a). Since each variant spreads over the arrays
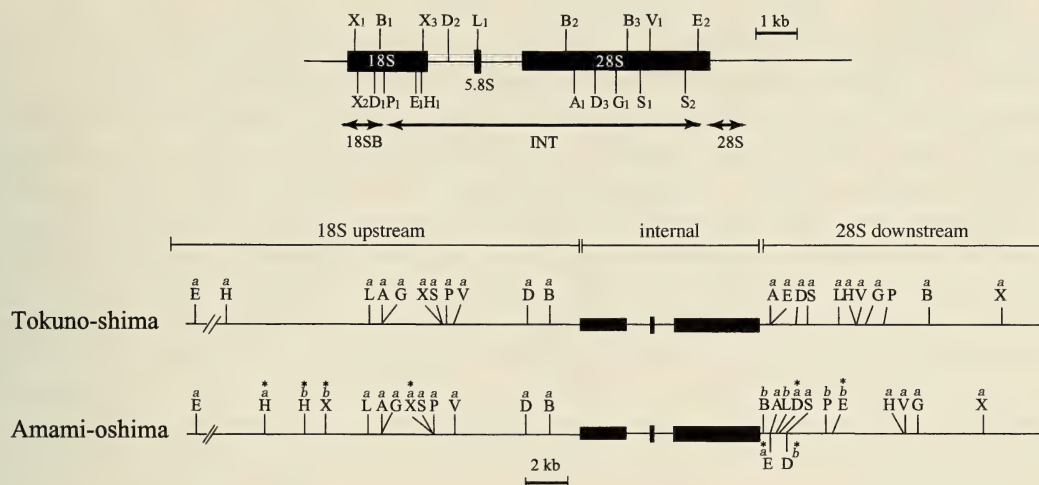
Fig. 1. Restriction maps of the rDNA repeating units of Tokuno-shima and Amami-oshima populations of *Tokudaia osimensis*. Each rDNA repeating unit is composed of three rRNA genes (28S, 5.8S, and 18S rRNA) which are separated from each other by spacers. With respect to the restriction sites on the flanking spacers, only those nearest to the distal end of the genes for 18S or 28S rRNA are shown. The upper diagram shows the conserved restriction sites in the coding and the internal spacer regions of the genes for 18S and 28S rRNA, which are not represented in the lower maps. The positions of the probes are also shown by arrows. Letters with superscripts represent specific types of restriction sites identified after comparison with the restriction maps. Types of Tokuno-shima are treated as *a*. Asterisks indicate polymorphic sites within individuals. A=*Aat*I; B=*Bam*HI; D=*Dra*I; E=*Eco*RI; G=*Bgl*II; H=*Hind*III; K=*Kpn*I; L=*Bcl*I; P=*Pst*I; S=*Sac*I; V=*Pvu*II; and X=*Xba*I.

of rDNA within a population through certain homogenization mechanisms (Coen et al. 1982), the presence of several distinct variants between the two islands clearly indicates that the two populations have been isolated for a considerable period of time. The amount of the sequence divergence, 2.3%, corresponds to a divergence time of 1.2–2.3 million years, if we assume that the divergence rate is 1–2% per million years (Suzuki et al. 1994a, 1999a).

Discussion

During this study we detected considerable differences in the cyt *b* sequences and the rDNA-RFLP between populations of *T. osimensis* from the islands of Tokuno-shima and Amami-oshima, as previously predicted by karyological analysis (Honda et al. 1977, 1978; Tsuchiya et al. 1989) and our preliminary molecular analysis (Tsuchiya et al. 1989). The difference between the populations, and the extent of the divergence in the two molecular markers, has greatly improved our knowledge of the evolutionary processes of these island populations. Our data will be helpful in assessing the evolutionary history and in reconsideration for taxonomic status of this species.

The extent of the inter-populational variation in the cyt *b* sequences between the two island populations was comparable to that between *Rattus* species and *Diplothrix legata* (Table 1). These results suggest that certain kinds of populational differentiation began a very long time ago. Differentiation of genes under the ordinal inherited mode, however, does not always reflect populational differentiation. Furthermore, in the case of mtDNA,

the differentiation patterns do not reflect the movement of males. For example, the differentiation patterns of mtDNA in geographically separate populations of the Japanese dormouse *Glirulus japonicus*, and Smithii's red-backed vole *Eothenomys smithii*, are not congruent with those of nuclear genes and morphological types (Suzuki et al. 1997, 1999a; Iwasa et al. unpublished). In contrast, data sets of the nuclear rDNA, a member of multigene families, would provide more useful information on the genetic status of given populations. The rDNA consists of several hundred copies within a genome, and a given variant extends to all of the units by certain homogenization mechanisms, and to all of the genomes of the same population as a result of mating (Dover 1980; Ohta 1980). In the case of *T. osimensis*, of the 22 restriction sites examined, three sites were completely differentiated, and four more were under differentiation between the rDNA repeating units of the two islands (Fig. 1). This data implies that the two island populations of *T. osimensis* have been reproductively isolated for some million years, despite there having been many chances to exchange genetic elements during the Pleistocene ice ages when falling sea levels led to land bridges existing between the islands (Kimura 1996). We could conclude, therefore, that these two island populations are already genetically differentiated to such an extent that there is little or no probability of future genetic contact. Consequently, the spiny rats from the two islands of Amami-oshima and Tokuno-shima may be better regarded as two independent species.

This assumption is congruent with the observed karyological differentiation between the island populations in which the diploid numbers are 2n=45 (Tokuno-shima) and 2n=25 (Amami-oshima). From the karyological perspective (Honda et al. 1977; Tsuchiya et al. 1989), such populations would not be expected to produce fertile progeny, that is they have been reproductively isolated through certain post-mating isolation mechanism. Such information clearly brings into question the current taxonomic status and suggests that the status of *T. osimensis* as a monotypic species should be reconsidered (Honda et al. 1977; Tsuchiya et al. 1989; Musser and Carleton 1993; Kaneko and Murakami 1996).

Interestingly, the extent of the cyt *b* divergence between these two island populations is somewhat similar to the level of distinctness of *D. legata* (Suzuki et al. 1999b; Table 1). This may imply that some geological event was attributable to both the geographical divergence of *T. osimensis* and to the migration and colonization of *D. legata* in the Okinawa Islands. The most simple explanation for such differentiation is that it was triggered by the disappearance of land bridges that once connected the islands of the region. Our rough time estimation predicts that divergence occurred 3.8–4.9 million years ago (Mya), taking into account the extent of transversional substitution (Table 1) and using the "standard" time estimation of the rat-mouse split as 12–14 Mya (though others have estimated the rat-mouse split to be more ancient (20–29 Mya, O'hUigin and Lee 1992; 40 Mya, Kumar and Hedges 1998)). If the time frame is estimated on the basis of a total-substitution rate of 2% per million years (Brown et al. 1979), then divergence is estimated to be 4.4 Mya. Both of these estimates related well to the geological view that the Ryukyu Islands were once connected to the Asian continent but became disconnected during the beginning of the Pleistocene, around 1.7 Mya (Kimura 1996). It may be postulated that such geological changes affected the differentiation of *D. legata* from other continental sister *Rattus* lineages, and simultaneously triggered the geographic divergence of the mtDNA haplotypes in *T. osimensis*.

In order to fully understand the various important issues related to the status of *T.*

osimensis, comparable data for *T. osimensis* from the Okinawa, is required. Karyologically, the Okinawan population represents the normal type, and may represent the ancestral situation of the unusual karyotypes. Studies of the Okinawan population are essential for the investigation of other issues such as the geographical differentiation of genes, and the taxonomic reconsideration of the island populations. Despite the scientific importance, all three populations of this taxon, especially in Okinawa, are thought to have already decreased to a point where those are seriously endangered possibly due to habitat destruction, predation by and competition with introduced species such as the Javan mongoose *Herpestes javanicus*, the feral cat *Felis catus* and the feral dog *Canis familiaris*, and the black rat *Rattus rattus*. The Environment Agency (1995) listed the Okinawan population as critically endangered and the populations of Amami-oshima and Tokuno-shima as endangered in the national Red List. Given the current status of *T. osimensis* in the wild in Okinawa, and given the remarkable biological importance of this taxon, effective conservation efforts are required, and these may include a research project to promote their reproduction in captivity while the issue of alien predators is dealt with.

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Apology and exchange

In acknowledging those persons who had kindly reviewed manuscripts for Vol. 23 (2) of *Mammal Study*, Dr. M. Motokawa's name was unintentionally omitted. I would like to deeply appologize to Dr. Motokawa for that oversight.

As there were also several mistakes in the index in Vol. 23 (2), please exchange it for the revised index attached to Vol. 24 (1).

Seiki Takatsuki (The former Chief in Editor)

EVOLUTION OF SHREWS

Edited by J.M. WÓJCIK and M. WOLSAN

In spite of an apparent recent increase of interest in shrews (Soricidae) and an impressive bibliography on the evolution of this fascinating group of mammals, until now there has been no comprehensive work that deals with current problems in shrew evolutionary research. This book represents an attempt to redress this omission, by offering a volume that seeks to review the present state of our knowledge of shrew evolutionary biology.

CONTENTS: Introduction; A Classification of the fossil and Recent shrews; Fossil history of shrews in Europe; Fossil history of shrews in Asia; Fossil history of shrews in Africa; Fossil history of shrews in North America; Dental adaptations in shrews; Chromosomal evolution in shrews; Chromosomal evolution: the case of *Sorex araneus*; Protein evolution in shrews; Mitochondrial DNA evolution in shrews; Evolution of energetic strategies in shrews; Evolution of social systems in shrews; Shrew mating systems; Appendix: A list of the living species of shrews; Taxonomic index.

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(2) 開催地に関わらず，公式言語が（通常）英語です．とはいっても，英語を母国語としている参加者ばかりではありません．「機関銃のような英語が飛び交うだろう」と尻込みしていては何も生まれません．度胸を決めて論議に参加することです．

(3) 事前の準備が早め．2－3年前からサーキュレーション（circulation 案内）が回されて仮登録が開始され，多くの場合1年以上前に登録が締め切られます．とはいっても，アブストラクトを提出しておけば良いだけで，データは直前までとれます．でも，会議後にプロシーディングス（proceedings 会議録）が出版される場合には，事前に原稿を提出する必要があります．

(4) 大きな会議では，セクションが設けられ，convenor とか chairman とか呼ばれる責任者をおいて，事前にテーマの設定，参加者の呼びかけ，会議の進行，プロシーディングスの編集など行うことがあります．

(5) 開催規模（参加人数）が大きく，期間が国内会議よりも長め．

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(7) エクスカーションもあるものが多い．同伴者のためのプログラムが用意されることもあります．

ければならないでしょう．ただし，日本学術会議との共同主催（年間8件）となれば，

(1) 国際会議委員会委員の会議出席旅費

(2) 国外へのサーキュラーの発送に関する経費

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(5) レセプション開催経費のうち，国外参加者相当分

に対する費用を予算の範囲内で負担されるようです．国際寄生虫学会では一千万円を申請したと聞いております．

　もう一点，重要なのが参加費です．アカプルコ大会では，「先進国の一般」，「途上国の一般」，「先進国の学生」，「途上国の学生」と4段階の格差が設けられ，それぞれ250米ドル，220米ドル，160米ドル，140米ドルでした．さらに早期割引と遅延割増の枠もあり，都合12段階の参加費が設定されたわけです．事務的には面倒ですが，参加者確保のための手段としては有効でしょう．募金や協賛金もさることながら，参加費も大きな財源になるためです．

3.　スケジュールの問題

　日本で開催するとなれば2005年（6年後）ですが，必ずしも時間的に余裕があるわけではありません．名古屋大会で承認が得られれば情報収集を開始し，1年以内に開催を引き受けるかどうかを決めなければなりません（2000年度の総会で決議し，年内に ITC 事務局に回答する必要があります）．また，日本学術会議との共同主催を考えるならば，2002年までに日程，会場，協力学会，資金計画などを固めておく必要があります（申請期限は開催の3年前）．セッションやワークショップのオーガナイズを予定している会員あるいは重要な事務的役割を担う会員は，2001年の ITC 大会（南アフリカ）に参加し，顔つなぎや情報収集を行なう必要があるでしょう．

4.　国際会議あれこれ
その意義，楽しみ，成果，教訓，失敗談，期待など

1.　国際会議とはどういうものか

　すでに参加を経験された方もまだの方もいらっしゃると思いますが，国際会議についてどういう印象や期待をお持ちでしょうか．ITC 日本開催を議論するに当たり，その意義や必要な心構えを大勢の人に考えてもらうために，若手・中堅の方々3人に，国際会議に出席して得た刺激・成果・教訓，また国際会議に対する期待などを10月に行われる哺乳類学会のミニシンポで話していただく予定です．事務局としては，21世紀の哺乳類研究の発展に向けて，ミニシンポを通じ一人でも多くの会員が ITC 開催を前向きに捉えてくれれば，と考えています．

　また，国際会議の運営に携わった経験をお持ちの方からもお話が聞けるといいと思いますが，残念ながらあまりいらっしゃらないようです（2005年には，大勢誕生しているかもしれません）．

2.　国内会議と何が違うのか

(1) 世界の哺乳類研究をリードしている多くの研究者が海外から参加します．論文や書籍でしか知らない哺乳類学のリーダーと出会える大きなチャンスです．自分の研究に対するアドヴァイスをもらえるかもしれませんし，また，海外調査や留学のきっかけを得られるかもしれません．

月にアカプルコで開かれる ITC で，次回の大会を日本で開けないかとの打診があると思われるが，日本哺乳類学会はどのように考えるか，と尋ねられました．当時，小原さんは ITC の運営委員をしておられ，その直前に ITC 事務局から開催の打診を受けたようです．

学会事務局は急いで，書面で評議員の意見を聴取しました．この結果，ほとんどの委員が，中堅・若手の飛躍のチャンスになる，学会のレベルアップにつながる，国際的な責任があるなどの理由で，ITC 開催を肯定的に考えていることがわかりました．しかし，組織的に力量不足であることの不安は拭えない，という意見も少なからずありました．

この時点で事務局は，①仮に開催を引き受けるにしても評議員会，総会を経る必要がある，②このような大問題を 1 度の総会で決定することも難しい，との判断から，4 年後の開催は時間的に不可能であると考え，アカプルコ大会で ITC 運営委員会に対して，「2001年大会の日本での開催は準備期間が短く，不可能である．将来の開催は真剣に検討しているが，すぐには答えられない」と返答することにしました．

アカプルコ大会後，評議員会で将来の開催問題について議論し，ITC 検討委員会を作り受け入れ可能な開催地について検討することにしました．検討委員会は東京，札幌，関西地区の開催が可能であることを答申し，さらに具体的な検討は評議員会が引き継ぐことになりました．

前後しますが，アカプルコ大会の直前に日本哺乳類学会に ITC 次回大会開催の打診があったのは，有力候補地の南アフリカ共和国が辞退すると申し出たためでした．このため，ITC 運営委員会はあわてて複数の代替地を探しまわったわけです．結局，南アフリカ共和国が辞退を撤回して，事なきをえました．

3. ITC 日本開催に関る事務上の諸問題

1. 協力学会等の問題

ITC が最も包括的な哺乳類学の大会であることを考えると，日本哺乳類学会が分野的に最も近い位置にあります．したがって，運営の基本姿勢に対する評議員会の考えは「他学会との協調を保ちながら本学会が主体的にアレンジを進める」という点に落ち着きました．ただし，「主体的」というのは「独占的」であることを意味しません．ITC の日本開催に興味を持つ学会も既にあると聞いていますし，前述のとおり ITC には研究分野の制限はありません．したがって，保全生物学，生態学，行動学，獣医学，畜産学，実験動物学等の関連学会と協力するのが最も当を得たものと考えています．また，他学会がセッション等のオーガナイズに参加すれば大会の幅が広がり，学術上も大きな意味があります．募金を集める上でも協力してもらえれば，財政的にも助かります．後述しますが，日本学術会議との共同主催も視野に入れるべき事項と考えられます．

2. 財政上の問題

哺乳類科学（38巻 2 号）の会記には「参加料収入の他に二千万円から三千万円の費用が必要」であることが報告されています．しかし，途上国に対する参加補助や招聘を充実すべきであること，円安傾向が続いていること（海外からの参加料が目減りする），日本の物価高などを考えると，この見積もりは最低ラインと考えた方が無難です．参考までに，1998年に開催された国際寄生虫学会では，約一億七千万円（参加費収入・国費補助の約九千五百万円を含む）を予定総額として募金活動を行ないました．ITC の参加人数や諸事情（国際寄生虫学会は医学系の学会であり，会場も幕張メッセを使った）を考慮すれば大幅な減額は可能でしょうが，数千万円を募金や協賛金で集めな

12. Large spatial and temporal scales in mammalian ecology: perspectives from the Americas

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1. Migratory bats: research and conservation priorities and perspectives

G. McCracken, R. A. Medellín

2. IUCN/SSC Lagomorph specialist group meeting   A. Smith, A. Velazquez, N. Formozov
3. US/Mexico cooperation in the conservation of rare species

W. Spencer, E. Mellink, J. Maldonado

4. History of mammalogy.   K. Sterling
5. Meeting of Latin American Mammal Societies   J. Arroyo, R. Ojeda
6. Prairie dog conservation   J. F. Cully
7. Meeting of the Mexican Society of Mammalogists   R. A. Medellín
8. Evolution of the Procyonidae   S. Zeveloff
9. Conservation issues concerning marine mammals in Latin America   S. Manranilla et al.
10. Mammal diversity and conservation in Latin America   G. Ceballos, R. A. Medellín
11. Ecology, evolution and conservation of Equidae   P. D. Moehlman

2. ITC の開催問題の経緯

1997年7月15日に女子栄養大学の小原秀雄さんから阿部永会長（当時）に電話があり，同年9

動物も含む）研究分野に制約はありません．4年に1度開催され，最近では欧米を中心に1,000人弱の参加者があると聞いています．これまでの開催地は 1. モスクワ，2. ブルノー，3. ヘルシンキ，4. エドモントン，5. ローマ，6. シドニー，7. アカプルコで，次回（第8回大会）は南アフリカ共和国で2001年に開かれる予定です．開催日数，プレナリーセッション数，シンポジウム数などはそれぞれの大会で違いがあったと思われますが，ここでは我々が参加したアカプルコ（メキシコ合衆国）大会の概要を紹介します．

1. アカプルコ大会

　アカプルコ大会は，1997年9月7日から11日までの5日間（3日目は休養日だったので実質は4日間）開かれました．シンポジウムは午前と午後に分かれて，主に半日ずつ（いくつかのセッションは午前から午後にわたって開かれた），それぞれ100人程度が参加できる会場で開かれ，午後のシンポジウム（ワークショップ）の前に大会場（参加者500人くらい）でプレナリーセッションがありました．また，ワークショップもシンポジウムとほぼ同じような扱いで開かれ，夜にはレセプションやバンケットがありました．これらの口頭発表に加え，ポスター発表も大きな会場で4日間あり，多くの若手（米国の大学院生が主体）で大変にぎわいました．プレナリーセッション，シンポジウム，ワークショップのタイトルとオーガナイザー（あるいは演者）は次の通りです．

Plenary sessions (4)

The Mexican commission of biodiversity: assessment of status of Mexican biological diversity

J. Soberon

The vicarious Gondwanan history of mammals, the other history　　　　R. Pascual

Body size and biodiversity　　　　J. H. Brown

The wild and the time in the past and the present　　　　J. Clutton-Brock

Symposiums (28)

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   S. T. Alvarez-C., L. Heaney

2. Mammal collections　　　S-T. Alvarez-C., A. Castro-C., M. Hafner

3. Canids ecology and conservation　　　T. Fuller, M. Mills, D. McDonald

4. Biology of gliding mammals　　　R. Goldingay, J. Scheibe

5. Global changes in mammal diversity at the end of the Pleistocene　R. Graham, J. Arroyo-C.

6. Demography and population dynamics in *Clethrionomys*

   L. Hansson, G. Bujalska, N. Yoccoz

7. Evolution and biology of Old and New World Hystricognath rodents　R. Honeycutt, Burda

8. Biology of subterranean rodents: evolutionary challenges and opportunities

   E. Lacey, G. Cameron, J. Patton

9. Biology and management of pest rodents　　　H. Leirs, G. Singleton

10. Ecology of disease and parasites in small mammals: victims and models

    H. Leirs, H. Henttonen

11. Behavioral and demographic responses to a patchy world: a mammalian perspective

    W. Lidicker

国際哺乳類学会（International Theriological Congress）の開催問題について

日本哺乳類学会事務局

1. 本冊子の送付理由

　経緯の詳細については前事務局長らが後述しますが，2005年に予定されている第9回国際哺乳類学会（International Theriological Congress；以下では ITC と略称する）の日本開催が日本哺乳類学会に打診されています．この件については，既に開催可能地域等の検討が行われて来ましたが，今年5月8日に臨時の評議員会を開き，さらに具体的な問題を論議しました．この結果，ITC の開催は，会員ならびに学会の海外交流を促進し，日本の哺乳類学の発展に大きく貢献すると判断し，「評議員会として受入れの方向で準備（現段階では実質的に情報収集）を始める」との結論に達しました．しかし，国際学会の開催は，大きな意義があるとはいえ，本学会は財政的・事務的に大きな負担を背負うことにもなります．したがって，「公式に準備を始める」ためには会員の了承を得る必要があることは言うまでもありません．次回 ITC 大会（2001年南アフリカ共和国）のスケジュールを考えると開催受け入れの「可否」は遅くとも来年中には公式に ITC 事務局に伝える必要があり，今年の名古屋大会では「本格的な準備を進めること」に関して会員の皆さんの理解をいただきたいと考えています．そのため評議員会は，名古屋大会の際に ITC 関連のミニシンポを開催し，ITC 開催問題の判断材料を会員に提供することを決めました．しかし，重要な問題なので数日の大会期間中に判断をしていただくには問題があります．そのため，事前にこの冊子を送付して総会時に承認をお願いする旨を予告し，さらに過去の ITC の内容や日本に対する開催打診の経緯，国際会議の開催に関わる財政的・事務的諸問題，学術的メリットなどを述べておくことにしました．ミニシンポとともに「学会として受入れの方向で準備を始める」ことに関する判断材料としてご活用下さい．なお，現段階で集まっている情報は，ほとんどが個人的に集められたものです（未承認のため「日本哺乳類学会」の名称を使った公的な情報収集は困難）．したがって，現実に沿わない部分や変更される部分が含まれている可能性があります．この点については，あらかじめご了承ください．

2. ITC と開催問題の経緯について

齊藤　隆（前事務局長）・中田圭亮（前・現会計幹事）

　ITC の開催問題は，1997年のアカプルコ大会の直前に ITC 事務局から打診されたことに端を発するので，当時事務局を務めていた齊藤隆と中田圭亮から同国際会議の性格と経緯のあらましを説明します．

　ITC は哺乳類学の最も包括的な国際会議で，野生哺乳類を対象としていれば（一部家畜・実験

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Subject

Instructions to contributors

Mammal Study publishes original *Articles*, *Short Communications* and *Reviews*, written in English, on all aspects of mammalogy. In principle, membership of the Society is pre-requisite for the submission of papers except for invited papers, but non-members may be co-authors. Manuscripts are submitted to qualified referees for critical scientific reviewing. Authors are notified, with referees' comments, on acceptance, rejection or need for revision within three months. The editor also customarily sends accepted manuscripts to qualified reviewers for English editing.

Manuscripts should be submitted typewritten on one side of the paper (use A4 21.0×29.7 cm paper), and double-spaced. An approximately 3 cm margin should be left on all sides. Do not hyphenate words at the right margin. Manuscripts should be arranged in the following order: the title, name(s) of author(s) and affiliation, fax number and E-mail address, abstract (fewer than 200 words) and key words (five words or fewer), main text, acknowledgments, references, tables, figure legends, figures. Titles of papers must be accurate and concise, and (for abstraction services) include any relevant taxonomic name. Text pages should be numbered through from title to references. Manuscripts should be line-numbered, every five lines, in the left margin. *Short Communications* do not exceed four printed pages. Abstracts and key words are omitted from *Short Communications*.

Tables and figures should be simple and self-explanatory, and their preferred locations should be indicated in the right margin of the text. The author's name and figure numbers should be written on the back of original figures and on the surface of copies.

Scientific names should be written in italic. All measurements should be in metric units. The following abbreviations should be used. Length: km, m, cm, mm, etc.; area: km^2, m^2, etc.; volume: km^3, m^3, kl, 1, ml, etc.; weight: kg, g, mg, etc.; time: hr, min, sec, etc.; others: cal, kcal, °C, Hz, *df*, *P* (probability), *SD*, *SE*, *CV*, etc. Arabic numerals should be used for numbers exceeding 10.

References in the text should follow the forms: "Uchida and Shiraishi (1985) stated that ...", (Abe and Kawamichi 1990), and (Miura et al. 1993). More than one reference within the same parentheses should be listed chronologically, alphabetically if of the same year. Full references cited must be listed alphabetically by the first author according to the following examples:

Abe, H., Shiraishi, S. and Arai, S. 1991. A new mole from Uotsuri-jima, the Ryukyu Islands. Journal of the Mammalogical Society of Japan 15: 47–60.

Eisenberg, J. F. 1981. The Mammalian Radiations. University of Chicago Press, Chicago, 610 pp.

Geist, V. 1982. Adaptive behavioral strategies. In (J. W. Thomas and D. E. Toweill, eds.) Elk of North America. Pp. 219–277. Stackpole, Harrisburg.

Obara, Y. 1991. Karyosystematics of the mustelid carnivores of Japan. Honyurui Kagaku [Mammalian Science] 30: 197–220 (in Japanese with English abstract).

Authors are recommended to refer to recent issues of the journal for details of style and layout.

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Original papers

Mammal
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The Continuation of *the Journal of*

the Mammalogical Society of Japan

The Mammalogical Society of Japan

The Mammalogical Society of Japan was founded in 1987 for the purpose of promoting and fostering mammalogy. The society publishes original papers in two journals: *Mammal Study* (the continuation of the *Journal of the Mammalogical Society of Japan*) for papers written in English, and *Honyurui Kagaku* [Mammalian Science] for those submitted in Japanese. Each journal is published twice a year. Submissions are considered on the understanding that they are being offered solely for publication by the Mammalogical Society of Japan. Both journals are distributed free of charge to the members of the Society. The following are the annual dues for the membership:

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Academic Society Center C21, 16-9 Honkomagome, 5-chome,
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Fax: +81(Japan)–3–5814–5820

Front cover: The red-backed vole with their chromosomes illustrated by Makiko Kashiwagi. Printed in Japan by Nakanishi Printing Co. Ltd., Kyoto.

Morphometric variation of house mice (*Mus musculus*) on the Izu Islands

Yasushi Takada[1], Eiichi Sakai[2], Yasushi Uematsu[3] and Takashi Tateishi[4]

[1,2,3]*Department of Anatomy, School of Dentistry, Aichi-Gakuin University, Chikusa-ku, Nagoya 464-8650, Japan*
[4]*Omiya Research Laboratory, Nikken Chemicals Co. Ltd, Kitabukuro, Omiya 330-0835, Japan*

Abstract.   We conducted univariate and multivariate statistical analyses of the morphometry of five island populations of the house mouse *Mus musculus*, from the Izu Islands (Oshima, Nijima, Kozushima, Miyakejima, Hachijojima), and compared them with three populations from the Japanese mainland of Honshu (from Kamogawa, Yokosuka, and Kawazu).   Analyses were based on bodies, mandibles and molars.   According to the analyses based on the mandible and molar measurements, the island samples differed from each other, and many of them also differed from the Honshu samples, although there was no evidence of positive directional variation, such as gigantism, in the insular samples.   Cluster analyses of morphological distance, based on mandible and molar measurements, indicated that the island populations, with the exception of that on Oshima, were closely related to those on Honshu, while the Oshima population was slightly more distantly related.   These results indicate that the divergence of the island populations is mainly attributable to the genetic variation of the initial founders and to subsequent isolation.   The differentiation of the island populations may have taken place as recently as within the past 1,200 years.

Key words: Izu Islands, mandible, molar, morphometric variation, *Mus musculus*.

Islands are physically isolated, and changes amongst island populations can be expected to be conserved and to progress rapidly.   Information concerning morphological and genetic changes in island populations is important, therefore, in contributing to an understanding of speciation.   Island populations of rodents have proven to differ morphologically and genetically from both mainland populations and from each other (see for example Hiraiwa et al. 1958; Miyao et al. 1968; Berry 1969; Berry and Rose 1975; Berry and Peters 1977; Berry et al. 1978; Sakai and Miyao 1979).   That these genetic differences can accumulate rapidly, has been shown by Berry and Jakobson's (1975) classic example of a mouse population on Skokholm Island, UK, which showed genetic changes over just 30 years.

The house mouse *Mus musculus* (or *Mus molossinus* according to Marshall and Sage (1981)), occurs on many of the small Japanese islands (Imaizumi 1960; Abe et al. 1994).   So far, genetic studies using biochemical markers have concentrated on the geographical variation amongst house mice throughout Japan (Minezawa et al. 1979, 1980), but they have not

[1]*E-mail: ytaka@dpc.aichi-gakuin.ac.jp*

found clear-cut divergences among insular populations, presumably due to the small number of specimens examined from each locality and because of the limited number of markers. Several studies have also revealed morphological variations amongst some insular house mice populations (Suzuki 1980; Takada et al. 1994), however, populations within an archipelago have not, so far, been studied.

The Izu Islands lie 90 km (Oshima) to 350 km (Aogashima) south of Tokyo Bay (Fig. 1). They range in size from Oshima, which at 91 km² is the largest, to Toshima and Shikinejima, which at just 4 km² are the smallest.   These islands are mostly inhabited, but have just five species of rodents and insectivores.   These are the house mouse, two species of rats *Rattus norvegicus* and *R. rattus*, the Japanese field mouse *Apodemus speciosus*, and the white-toothed shrew *Crocidura dsinezumi* (Nishikata 1986; Takada et al. 1999).   Both the field mouse and the house mouse occur together on Oshima, Nijima, Kozushima (Kozu) and Miyakejima (Miyake).   In addition, field mice alone are found on Shikinejima, and house mice are found alone on Hachijojima (Hachijo).   White-toothed shrews occur on Toshima, Nijima and Shikinejima.

In this paper, we describe the morphological differentiation of house mice in the Izu archipelago, having analyzed morphological variation amongst five insular populations from Oshima, Nijima, Kozu, Miyake and Hachijo.   Furthermore, we compare these five island

Fig. 1.   A map showing sampling localities.

populations with three mainland populations from Kamogawa (Boso Peninsula), Yokosuka (Miura Peninsula) and Kawazu (Izu Peninsula) areas of Honshu, the Japanese mainland.

Materials and methods

House mice were collected from December to March 1994 to 1997, using snap and live traps set in grassland around cultivated fields. Each island and peninsula was visited once for four to five days, with the exception of Kozu, which was visited three times in 1995 and 1996. The following data were recorded: sex, body weight (BW; for pregnant females, excluding embryos and uterus), head and body length (HBL; from rostrum to anus), tail length (TL; from anus to tail tip) and hindfoot length (HFL).

The age of the mice specimens collected was predicted using a linear regression equation of eye lens weight against age (after Takada 1985). Only data from adults (two months old and more) were analyzed for bodily dimensions.

Mouse heads were skinned and boiled for several minutes in water then soaked with a tripsin solution in order to produce clean skeletons (following Takada et al.'s (1994) technique).

The right mandibles and right molars were measured to the nearest 1/100 mm and 1/1000 mm, respectively, using a Nikon Measurescope. For mandible measurements, only specimens aged two to eight months old were used in order to reduce age-related variation (Lovell et al. 1984). The X-axis was fixed as the inferior edge of the mandible and the Y-axis as the anterior edge. Ten dimensions were measured; five (M1–M5) consisted of heights and the other five (M6–M10) of lengths (Fig. 2).

The buccolingual crown diameter was measured for upper (UM1 to UM3) and lower molars (LM1 to LM3). The occlusal surface was kept horizontal and the widest part of the crown was measured at right angles to the longitudinal axis of each molar; the axis being a line connecting the central cusps for upper molars, and the central groove for lower ones. For molar measurements, specimens with slightly worn cusps (dental wear categories 3 and 4 in Lidicker's (1966) criteria) were used, and an average of two measurements for each molar was used for the following analyses. Measurement errors were negligible; for the Oshima sample ($n=27$), σ_{errors} was $2.1 \cdot 10^{-3}$ to $3.1 \cdot 10^{-3}$ mm for each molar, where σ^2_{errors} was the sum of the squared difference of the two measurements divided by $2n$ (after Murai 1975).

Because no significant sexual differences ($P > 0.05$) were found (either for body (HBL,

Fig. 2. Diagram of right mandible showing the 10 measurements.

HFL, tail ratios=TL/HBL), or mandible or molar measurements; tested by *t*-statistics and Mann-Whitney's *U*- one TTEST and UTEST respectively (see Aoki 1995) for body, and using Wilks' *Λ*- one (WILKS, Aoki 1995) for mandible and molar measurements), data from both sexes were pooled for the following analyses.

Morphometric differences between samples were analyzed using univariate and multivariate statistics. Significant differences between sample pairs were tested using the Scheffé's method of multiple comparisons after Kruskal-Wallis test for the tail ratio, and using Ryan's method of multiple comparisons for the other variables (KWTEST and MCOMP respectively, Aoki 1995). To evaluate the divergence of the island samples, the relative deviation from the Yokosuka sample, one of the mainland samples with a relatively large number of specimens, was calculated using $(\text{mean}_1 - \text{mean}_2)/SD_2$, where, mean_1 was a sample mean, and mean_2 and SD_2 were the mean and SD of the Yokosuka sample. For multivariate statistics, principal component analysis (PCA, Tanaka et al. 1984), using a correlation matrix of pooled samples, was employed. Mahalanobis' generalized distance (D^2) was calculated as an indication of morphological divergence between samples (MAHPCV, Tanaka et al. 1984). Cluster analysis (CLUST, Tanaka et al. 1984) was also carried out using the group average method based on D^2.

Results

Body sizes

Significant differences between sample pairs were tested for HBL, HFL and tail ratios, but no positive directional change was found among insular and mainland samples. For sample means and *SD*s of adult mice see Table 1, and for a list of variables indicating significant differences between samples see the Appendix.

Table 1. Measurements for body size. For each measurement, sample means, *SD*, and number of specimens are given from the top.

	Oshima	Nijima	Kozu	Miyake	Hachijo	Kawazu	Kamogawa	Yokosuka
BW (g)	11.18	10.96	12.15	11.68	12.30	12.47	15.82	10.42
	2.67	1.53	2.94	1.91	1.94	2.02	2.28	2.37
	16	29	22	15	26	25	21	27
HBL (mm)	68.16	68.90	68.59	69.11	71.48	71.30	75.59	65.27
	5.86	4.82	6.21	4.82	5.29	5.40	4.42	5.36
	17	30	22	15	26	26	21	26
TL (mm)	55.89	57.66	56.00	59.51	62.02	60.71	59.42	55.14
	4.01	4.62	4.63	3.06	2.80	4.32	3.08	2.42
	15	30	22	15	26	25	22	27
HFL (mm)	15.35	15.63	15.28	15.23	15.67	15.74	15.74	15.46
	0.46	0.38	0.38	0.43	0.32	0.56	0.40	0.27
	17	30	22	15	26	26	22	27
TL/HBL (%)	82.15	83.85	81.81	86.29	87.03	85.09	78.43	85.00
	4.24	6.19	4.52	4.25	4.49	5.02	3.74	5.34
	15	30	22	15	26	25	21	26

BW: body weight; HBL: head and body length; TL: tail length; HFL: hindfoot length.

Mandible and molar measurements

Significant differences were found in variables between all pairs of sample means (see Table 2 for mandibles and Table 3 for molars).

Principal component analysis

The first component, expressing the overall size of the mandibles, arranges the samples from the largest, Kamogawa, to the smallest, Yokosuka and Miyake (see Table 4, Fig. 3). The second component, expressing the shape, particularly the height (M1, M2, M4; positive vector) to the length (M6, M7, M8; negative vector), arranges the samples from the highest, Oshima, to the lowest, Hachijo, Miyake and Kawazu. The third component, expressing relative height of the posterior (M3, M4, M5; positive vector) to anterior part (M1, M2;

Table 2. Measurements for mandible (×100, in mm). For each measurement, sample means, *SD*, and relative deviation from Yokosuka sample are given from the top. *n*, number of specimens.

		Oshima	Nijima	Kozu	Miyake	Hachijo	Kawazu	Kamogawa	Yokosuka
n		14	28	18	16	16	24	18	25
Age	mean	78	116	90	86	113	115	106	78
in days	*SD*	16	45	30	27	53	39	52	19
M1		164.3	165.4	155.4	156.6	155.4	154.9	165.7	152.6
		5.6	5.4	6.1	5.2	6.0	5.6	5.5	5.0
		2.32	2.54	0.56	0.79	0.56	0.45	2.60	0.00
M2		224.6	214.7	207.0	209.6	206.4	209.1	223.2	205.9
		7.8	9.3	9.4	8.8	9.5	9.4	8.8	7.6
		2.46	1.16	0.14	0.48	0.07	0.42	2.27	0.00
M3		434.4	452.3	457.3	428.9	446.8	442.9	455.2	433.9
		15.1	13.3	17.7	11.6	23.4	16.9	15.6	13.3
		0.04	1.38	1.76	−0.38	0.97	0.68	1.60	0.00
M4		476.3	476.6	481.0	441.3	475.9	468.3	489.0	465.1
		16.8	16.5	20.0	12.9	26.9	17.3	17.7	16.2
		0.69	0.71	0.98	−1.47	0.67	0.20	1.48	0.00
M5		541.2	546.5	556.2	520.8	544.9	550.9	559.5	530.1
		15.7	16.0	25.3	17.3	30.5	20.2	20.3	16.6
		0.67	0.99	1.57	−0.56	0.90	1.26	1.78	0.00
M6		722.5	742.6	741.2	755.4	757.5	762.9	767.6	729.8
		19.8	24.5	25.7	15.1	34.9	35.4	20.7	21.4
		−0.34	0.60	0.53	1.19	1.29	1.54	1.76	0.00
M7		781.8	795.5	793.9	797.4	798.9	829.6	818.2	773.8
		24.2	27.5	33.9	21.7	43.8	43.3	31.9	26.6
		0.30	0.82	0.76	0.89	0.94	2.10	1.67	0.00
M8		894.9	907.0	897.1	907.2	941.3	929.5	939.4	893.7
		25.6	28.8	33.8	20.4	41.0	32.9	23.6	24.4
		0.05	0.55	0.14	0.55	1.95	1.47	1.87	0.00
M9		1012.1	1004.8	991.9	1011.8	1047.1	1025.2	1054.1	987.7
		30.0	34.2	40.9	23.9	49.8	34.1	30.9	32.1
		0.76	0.53	0.13	0.75	1.85	1.17	2.07	0.00
M10		1047.5	1069.5	1068.2	1051.8	1086.9	1078.1	1103.3	1049.3
		33.9	36.1	47.4	23.3	44.1	43.9	31.6	33.5
		−0.05	0.60	0.57	0.07	1.12	0.86	1.61	0.00

Table 3. Measurements for molar ($\times 100$, in mm). For each measurement, sample means, *SD* and relative deviation from Yokosuka sample are given from the top. *n*, number of specimens.

	Oshima	Nijima	Kozu	Miyake	Hachijo	Kawazu	Kamogawa	Yokosuka
n	27	46	46	27	72	49	60	44
UM1	108.79	109.43	106.55	106.05	102.86	105.29	104.83	104.99
	1.81	1.99	1.49	2.25	2.04	2.30	2.22	2.11
	1.81	2.11	0.74	0.51	−1.01	0.14	−0.08	0.00
UM2	91.97	94.38	89.34	90.93	88.81	89.11	89.62	89.37
	1.95	2.18	2.24	1.97	1.90	1.80	2.47	2.19
	1.19	2.29	−0.01	0.71	−0.26	−0.12	0.11	0.00
UM3	61.15	64.99	63.02	63.62	64.60	60.61	62.50	61.83
	1.45	2.20	2.80	1.78	1.55	2.06	2.40	2.67
	−0.25	1.18	0.44	0.67	1.03	−0.46	0.25	0.00
LM1	87.59	87.37	85.18	82.98	81.44	83.89	83.84	82.40
	1.38	2.03	1.33	1.70	1.64	2.28	1.86	1.84
	2.82	2.70	1.51	0.31	−0.52	0.81	0.78	0.00
LM2	86.34	86.38	85.18	84.49	82.59	84.60	83.62	83.38
	1.40	1.60	1.01	1.89	1.73	1.75	2.20	1.77
	1.67	1.69	1.01	0.63	−0.45	0.68	0.13	0.00
LM3	54.86	57.84	59.98	58.92	57.64	56.13	56.85	56.68
	1.44	1.36	1.34	1.36	1.46	1.95	1.98	1.78
	−1.03	0.65	1.85	1.26	0.54	−0.31	0.09	0.00

negative vector), arranges the samples from the highest, Kozu, to the lowest, Miyake and Oshima.

The first component, expressing the overall size of the molars, arranges the samples from the largest, Nijima, to the smallest, Hachijo. The second component, expressing the relative size of the third molars (UM3, LM3; positive vector) to the first molars (UM1, LM1; negative vector), arranges the samples from the largest, Hachijo, to the smallest, Oshima. The third

Table 4. Principal component analysis of 10 mandible measurements based on pooled samples.

	Component		
Variable	1	2	3
M1	0.258	0.517	−0.380
M2	0.257	0.420	−0.533
M3	0.316	0.204	0.443
M4	0.299	0.316	0.450
M5	0.338	0.139	0.331
M6	0.327	−0.375	−0.141
M7	0.316	−0.332	−0.164
M8	0.340	−0.288	−0.075
M9	0.343	−0.164	−0.108
M10	0.351	−0.187	0.026
Eigenvalue	7.195	1.206	0.904
Proportion (%)	71.95	12.06	9.04
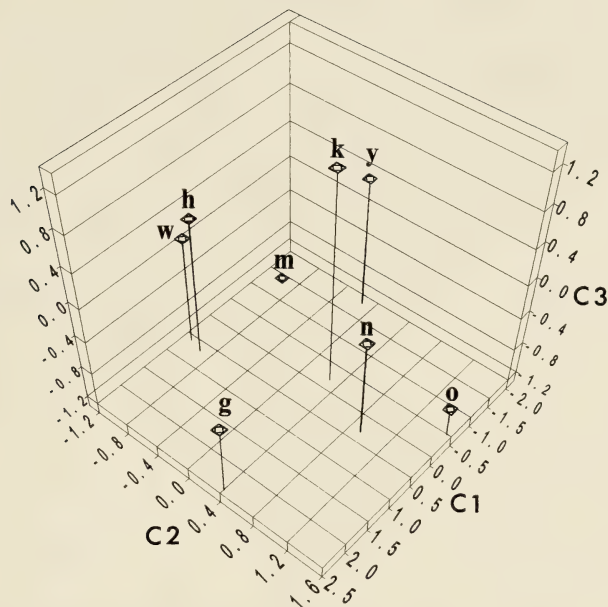
Fig. 3. Plots of sample means of the first three principal components, based on mandible measurements. g: Kamogawa; h: Hachijo; k: Kozu; m: Miyake; n: Nijima; o: Oshima; w: Kawazu; and y: Yokosuka.

component, expressing the relative size of the lower molars (LM2, LM3; positive vector) to the upper molars (UM2, UM3; negative vector), arranges the samples from the largest, Kozu, to the smallest, Oshima and Nijima (see Table 5 and Fig. 4).

Morphological differentiation

Morphological differentiation between samples was estimated from the Mahalanobis' distance (D^2) based on the mandible and molar measurements to be found in Table 6. The two sets of values are slightly different, but significantly correlated ($r=0.63$, $df=26$, $P<0.001$). The distance based on each character, i.e. mandibles and molars, indicates that many of the island samples differ from the mainland ones, whereas the mainland samples are

Table 5. Principal component analysis of 6 molar measurements based on pooled samples.

Variable	Component		
	1	2	3
UM1	0.478	−0.247	0.009
UM2	0.458	0.008	−0.511
UM3	0.266	0.659	−0.471
LM1	0.445	−0.333	0.126
LM2	0.473	−0.153	0.268
LM3	0.262	0.609	0.655
Eigenvalue	3.569	1.300	0.576
Proportion (%)	59.48	21.67	9.60
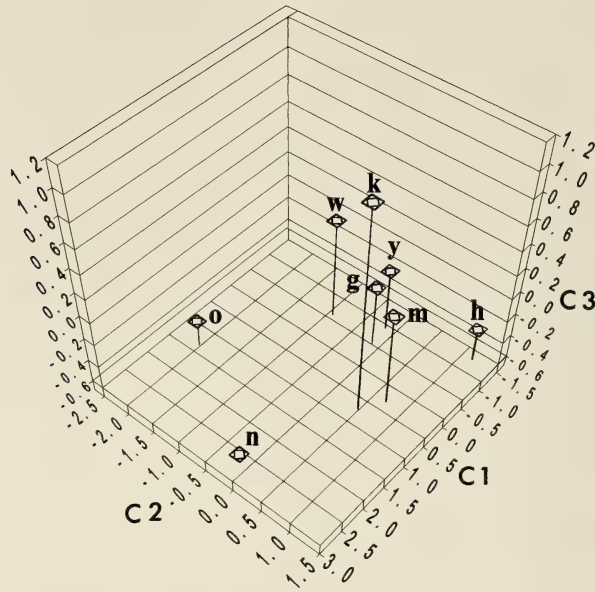
Fig. 4. Plots of sample means of the first three principal components, based on molar measurements. g: Kamogawa; h: Hachijo; k: Kozu; m: Miyake; n: Nijima; o: Oshima; w: Kawazu; and y: Yokosuka.

similar to each other. In contrast, the insular samples were found to differ greatly from each other (see Fig. 5).

The results of cluster analyses based on the two sets of the values are different (see Fig. 6). On the basis of their mandibles, three groups are identified, namely a) Oshima; b) Nijima, Yokosuka, Kamogawa, Kozu; and c) Miyake, Hachijo, and Kawazu. In contrast, on the basis of their molars, just two groups are identified, namely a) Oshima, Nijima; and

Table 6. Mahalanobis' distances (D^2) between samples using 10 mandible and 6 molar measurements. For each pair-samples, the values from mandible and molar are given in the upper and lower lines respectively.

	Oshima	Nijima	Kozu	Miyake	Hachijo	Kawazu	Kamogawa
Nijima	24.05						
	7.20						
Kozu	39.51	17.53					
	26.36	14.95					
Miyake	50.49	25.58	37.65				
	24.99	11.44	4.47				
Hachijo	41.53	24.32	29.92	13.54			
	33.74	19.21	9.89	4.86			
Kawazu	38.46	24.19	15.13	18.56	11.67		
	9.38	9.16	8.54	6.71	11.15		
Kamogawa	12.26	9.07	21.97	20.76	13.85	17.17	
	14.21	7.33	5.95	3.72	4.93	2.36	
Yokosuka	21.70	13.78	9.00	22.80	13.24	9.16	8.51
	17.37	10.27	7.03	2.05	5.13	2.59	1.49

All the values are significantly different from zero ($P < 0.002$).
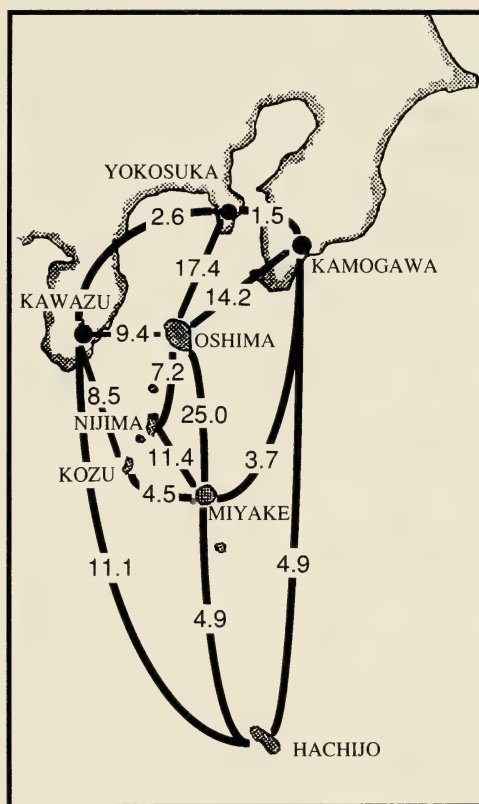
Fig. 5. Mahalanobis' distances (D^2) between samples, based on molar measurements.

b) all of the others.

Discussion

It is necessary to examine factors affecting size, such as sex, growth and so on, before comparing variations among populations on the basis of morphometrics (Thorpe 1981). In this study, only characteristics that did not differ significantly between the sexes were analyzed. Many bodily dimensions increase after reaching sexual maturity, whereas hind-foot length and tail ratio increase only slightly (Hamajima 1963). Similarly, the shape of the mandible is nearly constant after seven weeks of age, although the overall size increases slightly (Lovell et al. 1984). In addition, the enamel of the molar crowns does not change in size and shape after reaching full size, without there being severe wear to the cusps (Sakai 1989). For this analysis, only external body and mandible measurements from adult mice were used. Molar size is a very useful characteristic for use in morphometric comparisons because the size of the crown changes very little, and because its heritability is relatively high (Leamy 1974; Murai 1975).

Differences were found in both mandible and molar measurements between mice from the various islands in the Izu archipelago, however difference in bodily dimensions were not so conspicuous. The variations were not, however, directional, and there was no evidence of
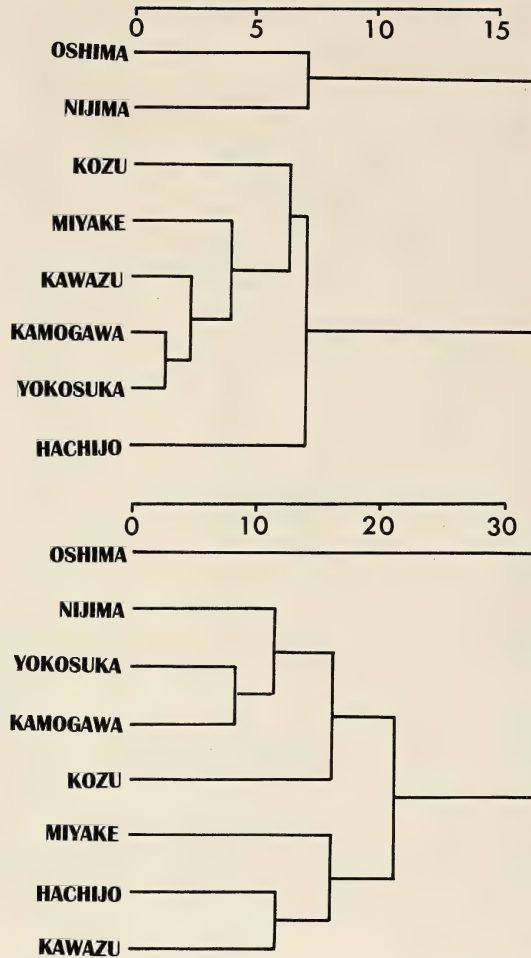
Fig. 6.   Cluster analyses based on molar (upper) and mandible measurements (lower).

the gigantism amongst insular mice and insectivores as reported, for example, by Foster (1964) and Miyao (1970).   Bodily dimensions (HBL, HFL, TL/HBL) of the insular samples ranged within those of the mainland samples (Table 1 and Appendix).   Similarly, the overall mandible size of the insular samples, judging from the first component of PCA, ranged within that of the mainland samples (Fig. 3).   Some insular samples (Oshima, Nijima) had larger molars, whereas specimens from Hachijo had the smallest molars, judging from the first component (Fig. 4).

Morphological differentiation was greater among most of the island populations than among the mainland ones, as indicated by the Mahalanobis' distance (Table 6 and Fig. 5). Most of the distances between the island samples exceeded those between the mainland ones (islands: 13.54–50.49 for mandibles, 4.47–33.74 for molars; mainland: 8.51–17.17 for mandibles, 1.49–2.59 for molars).   It seems that differentiation among the island populations were unrelated to the geographical distance separating them.   For example, although the islands of Nijima and Kozu are only 15 km apart, their mice populations are well differentiated from each other.   Furthermore, many of the island populations have also diverged from

the mainland populations, although this was more conspicuous when examining molars than mandibles (Table 6 and Fig. 6). The Oshima population (on the basis of its mandibles and molars) and the Nijima population (on the basis of its molars) were particularly well differentiated from the mainland populations, according to cluster analyses based on the Mahalanobis' distance. In contrast to these cases, some island populations were closely related to some of the mainland populations on the basis of their mandibles or molar measurements. In the case of mandible measurements, the Kozu population was similar to the Yokosuka population, Nijima to Kamogawa, Hachijo to Kawazu. In the case of molar measurements, the Miyake population was similar to the Yokosuka population.

In general, insular mice and insectivores diverged both morphologically and genetically. Japanese field mice, *Apodemus speciosus*, *A. argenteus*, and shrew-moles, *Urotrichus talpoides*, on the Oki Islands differ from island to island (Dogo, Dozen), in body, skull and molar sizes (Hiraiwa et al. 1958; Miyao et al. 1968; Sakai and Miyao 1979; Uematsu 1993; Sakai et al. 1997a, b). Similarly, voles and mice in the British Isles differ both morphologically and genetically among adjacent islands, i.e. Orkney voles, *Microtus arvaris* on the Orkney Islands (Berry and Rose 1975), house mice, *Mus domesticus* on the Faeroe Islands (Berry and Peters 1977; Berry et al. 1978; Davis 1983), and wood mice, *A. sylvaticus* on the Hebridean and Shetland Islands (Berry 1969).

The degree of the divergence found in the house mice on the Izu Islands matches that found in the field mice and shrew-moles on the Oki Islands, judging from an index of the relative deviation from a mainland sample. The maximum value of the index for molar sizes (buccolingual crown diameters) ranged from 1.03 to 2.82 in the present results (Table 3). The relevant figures are 2.32 and 3.50 for *A. speciosus* (Sakai and Miyao 1979), 0.89 and 1.95 for *A. argenteus* (Sakai et al. 1997b) and 0.5, 2.5 and 3.0 for shrew-moles (Sakai et al. 1997a).

It seems that insular populations are likely to differentiate from each other over rather short periods of time. Examples among rodents have been reported from the Orkney and Guernsey voles, three subspecies of *M. arvalis* (Berry and Rose 1975), and in 15 subspecies or races of wood mice, *A. sylvaticus*, on the Hebridean and Shetland Islands (Berry 1969). Berry and Rose (1975) suggested that the Orkney vole was brought to the islands by man about 4,000 years ago, and Berry (1969) considered that the Hebridean and Shetland groups of mice were introduced by the Vikings or their descendants. Another example comes from among the populations of house mice in the Faeroe Islands, some of them were probably founded less than 200 years ago, yet nevertheless, these newer populations are as distinct as the older ones (Berry et al. 1978). Berry (1981) considered these to be examples of instant sub-speciation.

On the basis of the geological evidence, and from data relating to sea-level changes, it seems that the Izu Islands have never been connected to the Japanese mainland by land (Taira 1990; Shimizu 1996). House mice are presumed to have been inadvertently transported to the islands in ships carrying cargo. If so, it is likely that the island populations were founded by small numbers of mice from the mainland, with perhaps some later re-colonization.

The divergence of the mouse populations on the Izu Islands might have arisen either from natural selection after colonization and isolation, or from the genetic variants among the founders. Any change due to the latter is stochastic, and is known as the founder prin-

ciple (Williamson 1981).   As no directional change was found among these insular popula-
tions, it seems most likely that they have diverged as a result of the founder principle.   The
subsequent divergence may be ascribed to natural selection working on the original genetic
traits of the founders.   Repeated colonization of the islands by mice from the mainland or
other islands may have occurred in accordance with recent increases in traffic.   Colonization,
however, may not always have been successful (Berry et al. 1982).   Re-colonization by large
numbers of mice may accelerate both hybridization with an initial population and genetic
and morphological differentiation, that processes were analyzed experimentally by Berry et
al. (1991) and Scriven and Bauchau (1992).

The combination of isolation and history are very important contributory factors to the
divergence of island populations.   A good example can be found among the house mice on
Chichijima, in the Ogasawara Islands.   Their existence on the island is probably associated
with the human colonization that took place from Hachijo and Hawaii during the 19th
century (Takada et al. 1994).   On the basis of their genetic traits, the mice on Chichijima
seem to be hybrids between *Mus musculus molossinus* and *M. m. domesticus* (Moriwaki et
al. 1984; Miyashita et al. 1985; Yonekawa et al. 1988), and they appear unique both in their
overall morphology and specifically in their mandibles (Tateishi and Takada 1994; Takada
et al. 1994).   Isolation on the island probably accelerated the hybridization.   Furthermore,
isolation combined with colonization by a small number of mice has probably been the cause
of very low genetic variation within individual insular populations of mice (Berry and Peters
1977; Berry 1981).

The earliest human traces on the Izu Islands date back to the Paleolithic Period (up to
about 20,000 B. P.), although confirmed evidence of human settlement in the islands dates
to the Jomon (up to about 10,000 B. P.) and Yayoi periods.   Many of the remains have been
recorded since the 8th century (the Nara Age), indicating regular traffic between the islands
and the nearby mainland, especially the Izu Peninsula (Hashiguchi 1988; Nijimamura 1996).
In more modern times (the Edo Age), commerce prospered and many ships from various
parts of Japan were wrecked on the islands (Hachijocho-Kyoiku-Iinkai 1973; Nijimamura
1996).   Unfortunately, there is no reliable information on the timing of the colonization of
the islands by the house mouse, though it may have been during the 8th century when there
was frequent freight traffic to and from the mainland, or during the 17th and 18th centuries
when agriculture prospered (Hachijocho-Kyoiku-Iinkai 1973; Danki 1976).

The house mice populations currently living on the Izu Islands are clearly related to
those of the Japanese mainland.   Some island populations, such as that on Oshima, differ
slightly from the others, indicating that they may have a different genetic background.   In
order to understand the nature of the differentiation between the populations, and to
ascertain the origins of the island mice populations, it is necessary to know their genetic
traits and to synthesize these with their morphological variation.

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Appendix. Statistical table for body size-variables indicating significant differences between pairs of samples (tested by Ryan's method of multiple comparisons for H, F and Kruskal-Wallis test for T; $P=0.05$).

	Oshima	Nijima	Kozu	Miyake	Hachijo	Kawazu	Yokosuka
Nijima							
Kozu		F					
Miyake		F					
Hachijo			F	F			
Kawazu			F	F			
Yokosuka					H	H	
Kamogawa	H	H	H,F	H,F,T	T	T	H,T

H, HBL; F, HFL; T, TL/HBL.

Morphometric status of shrews of the *Sorex caecutiens/shinto* group in Japan

Nikolai E. Dokuchaev[1], Satoshi Ohdachi[2] and Hisashi Abe[3]

[1]*Institute of Biological Problems of the North, Magadan 685000, Russia*
[2]*Institute of Low Temperature Science, Hokkaido University, Sapporo 060-0819, Japan*
[3]*Katsuraoka 26-17, Otaru 047-0264, Japan*

Abstract.   The morphometric relationships among five operational taxonomic units of the *Sorex caecutiens/shinto* group (Soricidae) (*S. caecutiens* of Hokkaido, *S. shinto shinto* of Honshu including the *S. chouei* holotype, *S. s. shikokensis* of Shikoku, and *S. s. sadonis* of Sado) in the Japanese Islands, were examined using uni- and multivariate analyses of 15 cranial, dental, and external characters.   The morphological analyses showed that the shrew from Hokkaido (*S. caecutiens*) and those from Honshu, Shikoku, and Sado (*S. shinto*) were exclusively differentiated.   In particular, the surface structure of the fourth upper premolar completely separated the two taxa.   In contrast, *S. s. sadonis* from Sado could not be completely distinguished from related taxa from Honshu and Shikoku.   Thus these morphometric analyses re-confirm that *S. caecutiens* of Hokkaido, and *S. shinto* from Honshu, Shikoku, and Sado, should be treated as two separate species, as has previously been proposed on the basis of a molecular phylogenetical study.

Key words: *Sorex caecutiens*, *S. shinto*, *sadonis*, *shikokensis*, taxonomy.

Thomas (1905, 1906) described *Sorex shinto* as a new shrew species from Honshu Island. Later, he described a new subspecies, *S. shinto saevus*, from Sakhalin Island and included the island of Hokkaido in its distributional range (Thomas 1907).   Thomas (1907) did not find any morphological specific differences, however, among the shrews from Honshu, Hokkaido, and Sakhalin Islands.

In the years since Thomas's (1907) study, the taxonomic treatment of *S. shinto* and other taxa of the *Sorex caecutiens/shinto* group (in the sense of Ohdachi et al. 1997a) has varied. Bobrinskii et al. (1944) treated some medium-sized shrews from Eurasia, including *S. shinto* described by Thomas (1907), as a single species, *S. macropygmaeus* Miller, 1901.   Ellerman and Morrison-Scott (1951) accepted Bobrinskii et al's (1944) systematic concept, but they synonymized *S. macropygmaeus* with *S. caecutiens* Laxmann, 1788.   Stroganov (1957), who investigated the shrews from Sakhalin, Hokkaido, and the southern Kurile Islands in great details, concluded that the shrews in Hokkaido and Sakhalin, described as *S. shinto saevus* by Thomas (1907), should be included in *S. caecutiens*, as Ellerman and Morrison-Scott (1951) did.   However, without inspecting *Sorex* samples from Honshu, Stroganov (1957)

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considered that the shrews of Honshu were a subspecies of *S. caecutiens*, *S. c. shinto*.

Since Stroganov's (1957) investigation, there have been two main opinions concerning the taxonomic status of *shinto* in the *caecutiens/shinto* group.   On the one hand, some authors have objected to the specific rank of *shinto*, and have followed Stroganov (1957) in including it in *S. caecutiens* (e.g. Bobrinskii et al. 1965; Abe 1967, 1994; Gureev 1971, 1979; Yudin 1971, 1989; Corbet 1978; Gromov and Baranova 1981; Krivosheev 1984; Dolgov 1985; Okhotina 1993; Dobson 1994).   Furthermore, with regard to the subspecific status of the *caecutiens/shinto* shrews of the Japanese Islands and vicinity, Abe (1967, 1994) treated the population of Honshu as *S. caecutiens shinto*, that of Shikoku as *S. c. shikokensis*, and that of Hokkaido and Sakhalin as *S. c. saevus*.   On the other hand, some authors have been of the taxonomic opinion that *S. shinto* should be considered as an independent species that occurs in Honshu, Shikoku, and Hokkaido (and Sakhalin, according to some authors) while *S. caecutiens* across the Eurasian Continent (and in Sakhalin according to some authors), essentially following Thomas's (1907) position (e.g. Imaizumi 1949, 1960; Sokolov 1973; Yoshiyuki and Imaizumi 1986; Pavlinov and Rossolimo 1987; Hutterer 1993; Pavlinov et al. 1995; Wolsan and Hutterer 1998).   In addition, Imaizumi (1954) described *S. chouei* from Honshu as a new species, although this was later synonymized with *S. caecutiens* (Abe 1967, 1994, 1996) or *S. shinto* (Imaizumi 1970; Hutterer 1993).

There has been the additional controversy in Japan concerning the taxonomic status of the Sado shrew as part of the *caecutiens/shinto* group.   This taxon was first described by Yoshiyuki and Imaizumi (1986) from Sado Island.   While some authors treat it as an independent species, *S. sadonis* Yoshiyuki *et* Imaizumi, 1986 (e.g. Abe 1994, 1996; Wolsan and Hutterer 1998), others suggest that it should be considered a subspecies of *S. shinto*, *S. s. sadonis* (Ohdachi et al. 1997a; Koyasu 1998).

Ohdachi et al. (1997a) recently used the DNA sequences of the mitochondrial cytochrome *b* gene to reveal the phylogenetical relationships among northeastern Asiatic soricine shrews.   Their work indicated that all of the shrews from Honshu, Shikoku, and Sado should be considered as belonging to a single species, *S. shinto*, whereas those from Hokkaido and Sakhalin, belonged to the widespread Eurasian continental species, *S. caecutiens*.   This taxonomic scheme has subsequently been followed by Koyasu (1998).   No investigations have been made, however, of the morphological relationships among the local populations (or subspecies) of *S. caecutiens* and *S. shinto*.

Our goal was to reveal the morphological status of the *Sorex caecutiens/shinto* group in the Japanese Islands (Hokkaido, Honshu, Shikoku, and Sado).   As a result of this research, we are able to offer a morphological diagnosis making it possible to distinguish *S. shinto* from *S. caecutiens* (in the sense of Ohdachi et al. 1997a and Koyasu 1998).

Materials and methods

We have followed the taxonomic approach of Ohdachi et al. (1997a) and Koyasu (1998) for the *caecutiens/shinto* group, and call the shrews of Hokkaido *S. caecutiens*, those of Honshu (including *S. chouei* Imaizumi, 1954) *S. shinto shinto*, those from Sado *S. s. sadonis*, and those from Shikoku *S. s. shikokensis*.   These five operational taxonomic units (OTUs) were used for the present investigation.   As to geographical terms, we refer the total area of Honshu, Shikoku, and Sado Islands to the "Honshu complex".

Specimens of the *Sorex caecutiens/shinto* group at the National Science Museum (Tokyo), the Natural History Museum, Faculty of Agriculture (K. Maekawa and H. Abe collections), and the Institute of Low Temperature Science (S. Ohdachi collection), Hokkaido University (Sapporo), were examined to provide the basic data for this study. Undamaged skulls of 40 *S. caecutiens* from Hokkaido, 45 *S. shinto* from Honshu (including the holotype of *S. chouei* Imaizumi, 1954, specimen code NSMT-M12513), one *S. s. shikokensis* from Shikoku (the holotype of *S. caecutiens shikokensis* Abe, 1967, NHMHU-13311), and six *S. s. sadonis* from Sado (including the holotype of *S. sadonis* Yoshiyuki *et* Imaizumi, 1986, NSMT-M16180) were used for the cranial and dental analyses, and 240 specimens of *S. caecutiens* from Hokkaido and 25 *S. shinto* from Honshu were used for the analysis of external characters. Specimen codes and locations are listed in the Appendix. Only young-of-the-year (=sexually immature) specimens were used for the cranial and external measurements, with the exception of the three holotype specimens, all of which had over-wintered (=sexually matured). The reason for choosing primarily immature shrews was that the skulls of the over-wintered shrews tend to be slightly smaller (Ognev 1933; Stroganov 1957; Abe 1967), their teeth may be worn, whereas their external characters, such as body length and body weight, tend to be much greater. The three holotypes were used only for the cranial analyses. Samples from both sexes were pooled for analysis, since there is no significant difference in skull size between males and females of the *caecutiens/shinto* group (Abe 1967).

Nine cranial and dental characters were measured. Definitions for these characters are as follows. 1) Condylobasal length: the length from the anterior medial point of the pre-maxillary bone to the posteriormost point on the occipital condyle. 2) Facial length: the length from the anterior medial point of the premaxillary bone to the posteriormost point of the foramen on the frontal bone. 3) Breadth of the braincase: the maximum width of the braincase. 4) Glenoid width: the maximum width between the right and left mandibular fossae. Definitions for (3) and (4) are illustrated in Dannelid (1994). 5) Width across the second upper unicuspids: the width between the outer margins of the right and left second upper unicuspids (U^2) viewed from the crown side. 6) Width across the second upper molars: the width between the outer margins of the right and left second upper molars (M^2) viewed from the crown. 7) Length of the upper molariform tooth row: the length from the anterior point of the fourth upper premolar (i.e. the superficial "third" premolar) to the posterior point of the third molar, viewed from the crown. 8) Length of upper unicuspid row: the length from the anterior point of the first unicuspid to the posterior point of the fifth unicuspid, viewed laterally. 9) Relative basal width of the mesostyle of the fourth upper premolar: length from the anterior point of the fourth upper premolar (Pm^4) to the posterior point of the mesostyle ("a–b" distance in Fig. 1) relative to Pm^4 length ("a–c" distance), expressed in percentage ("a–b"/"a–c" × 100). Here, we have followed Stroganov's (1957) and Dolgov's (1985) terminology for tooth anatomy.

Skull and tooth characters were measured using an ocular micrometer under a binocular microscope, with the exception of the condylobasal length, which was measured using callipers. Most characters were measured to the nearest 0.01 mm, however condylobasal length was measured to the nearest 0.1 mm. The relative width of Pm^4 mesostyle was measured, using digitally-saved images from a photo-capturing system: OLYMPUS micro-scope (SZH10), OLYMPUS-Ikegami CCD camera (ICD-740), and a Macintosh computer
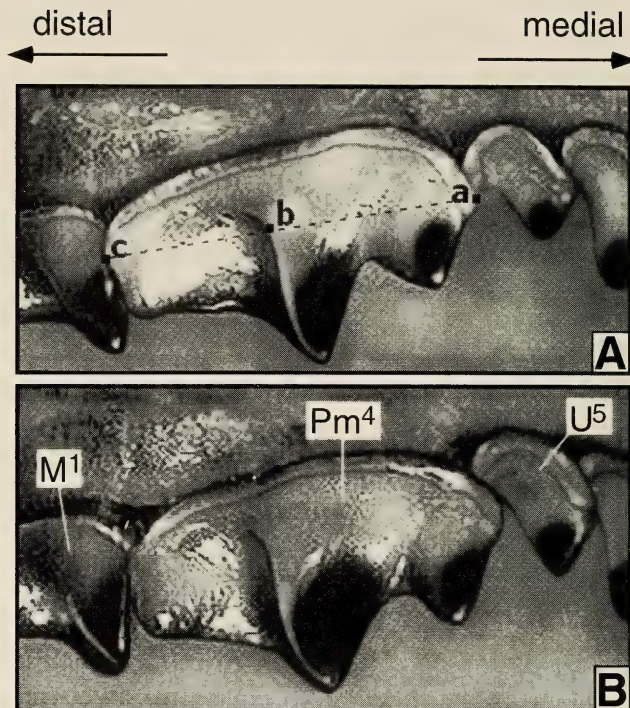
Fig. 1. Buccal view of the fourth right upper premolar of (A) *Sorex shinto* from Honshu (specimen code, SO-96misc15) and (B) *S. caecutiens* from Hokkaido (SO-88n105). Pm⁴, fourth upper premolar; M¹, first upper molar; U⁵, fifth unicuspid.

(Performa 5430).

Six external characters, body weight, total body length, tail length, hind-foot length, tail ratio, and hind-foot ratio, were used in the analyses. Measurements of the external characters were obtained from the original specimen labels, once doubtful data has been carefully eliminated. Data from both sexes were combined, since there are no sexual differences in the external characters of young shrews in the *caecutiens/shinto* group (Abe 1967). For our purposes, the tail ratio was calculated as the percentage tail length to head and body length, and the hind-foot ratio was the percentage to tail length.

Multivariate factor, cluster, and discriminant analyses were carried out, using the nine cranial characters. For cluster analysis, the nearest neighbor method using Euclidean distance was applied. Differences in means of cranial and external characters between *S. caecutiens* in Hokkaido and *S. shinto* in Honshu were tested using Student's *t*-test for most characters and Mann-Whitney's *U*-test for ratios (relative width of Pm⁴ mesostyle, relative tail length, and relative hind foot length).

Results

The cranial characters of the shrews from Hokkaido (*S. caecutiens*) were found to be significantly larger than in those of the shrews from Honshu (*S. s. shinto*) (Table 1). Remarkable differences between these two taxa were found in the relative basal width of

Table 1. Means±1*SE*, ranges (in parentheses), and the results of *t*- and *U*-tests of cranial characters in *Sorex caecutiens* from Hokkaido Island and *S. s. shinto* from Honshu Island. All the specimens were the young animals. *U*-test was conducted for relative basal width of Pm⁴ mesostyle, and *t*-tests for the other characters.

Cranial and dental characters	*S. caecutiens* in Hokkaido ($n=40$)	*S. s. shinto* in Honshu ($n=44$)	*t* or *U*-test	
			t or *U*	*P*
Condylobasal length (mm)	18.0±0.05 (17.0–18.5)	17.4±0.06 (16.5–18.1)	7.75	<0.001
Facial length (mm)	9.02±0.033 (8.55–9.55)	8.70±0.051 (8.12–9.35)	5.10	<0.001
Breadth of braincase (mm)	9.17±0.031 (8.80–9.60)	8.73±0.034 (8.32–9.30)	9.30	<0.001
Glenoid width (mm)	5.04±0.021 (4.70–5.30)	4.78±0.022 (4.55–5.15)	8.38	<0.001
Width across U² (mm)	1.81±0.009 (1.70–1.95)	1.73±0.012 (1.50–1.90)	5.56	<0.001
Width across M² (mm)	4.23±0.016 (4.05–4.50)	4.11±0.020 (3.85–4.40)	4.73	<0.001
Length of upper molariform tooth row (mm)	4.35±0.014 (4.20–4.60)	4.20±0.017 (3.95–4.42)	6.68	<0.001
Length of upper unicuspid row (mm)	2.73±0.011 (2.55–2.85)	2.49±0.012 (2.34–2.65)	15.30	<0.001
Relative basal width of Pm⁴ mesostyle (%)	61.2±0.20 (59.3–65.2)	54.9±0.20 (52.1–57.2)	272.0	<0.001

Pm⁴ mesostyle and the length of the unicuspid row (Table 1). While almost all craniometric characters overlapped between the two taxa, no overlap was found in observed values of the relative width of the Pm⁴ mesostyle (Table 1). The shrews from Hokkaido were heavier, and had longer hind feet than the shrews from Honshu, but they did not differ in their total body length (Table 2).

The shrews of Hokkaido could not be distinguished from the shrews of the Honshu complex (*S. shinto* sspp.) on the basis of the first rotated factor of the factor analysis of the craniometrical characters (Fig. 2), and the average first factor value of Hokkaido shrews was intermediate between those of the shrews from Honshu, and the shrews from Sado and Shikoku (*S. s. sadonis* and *S. s. shikokensis*) (Fig. 2). The second rotated factor, however, clearly distinguished between the shrews of Hokkaido and of the Honshu complex (Fig 2). The second rotated factor was greatly contributed to by the relative width of the Pm⁴ mesostyle, as well as the length of upper unicuspid row (Table 3).

Cluster analysis showed that the Hokkaido shrews are distant from the shrews of the Honshu complex, which occur in a closely related single cluster (Fig. 3). Within the cluster for the Honshu complex, the shrews from Sado and Shikoku formed a secondary cluster.

According to discriminant analysis, five out of the nine characters were significant enough to be able to distinguish between the shrews of Hokkaido and of the Honshu complex. The discriminant function between the two shrew groups was as follows:

Table 2.   Means±1*SE*, ranges (in parentheses), and the results of *t*- and *U*-tests of external characters in *Sorex caecutiens* from Hokkaido Island and *S. s. shinto* from Honshu Island.   These specimens were all of young animals. *U*-tests were conducted for the two characters of ratio, and *t*-tests for the other characters.

External characters	S. caecutiens in Hokkaido (n=240)	S. s. shinto in Honshu (n=25)	t or U–test	
			t or U	P
Weight (gram)	5.0±0.03	4.4±0.11	5.55	<0.001
	(4.0–6.7)	(3.5–5.9)		
Total body length (mm)	113.8±0.25	112.6±0.91	1.36	ns*
	(98–126)	(108–125)		
Length of tail (mm)	48.2±0.22	50.7±0.55	3.52	<0.01
	(40.0–58.0)	(46.0–56.5)		
Length of hind foot (mm)	12.4±0.03	12.0±0.08	4.84	<0.001
	(11.1–13.5)	(11.2–12.9)		
Tail ratio to head & body length (%)	73.8±0.44	82.4±1.84	1392.0	<0.001
	(60.0–103.6)	(66.2–98.2)		
Hind-foot ratio to tail length (%)	25.8±0.11	23.7±0.30	1065.5	<0.001
	(21.5–30.5)	(21.7–26.4)		

* *P*>0.05.

□ *S. s. shinto* in Honshu   ★ *S. s. shinto* (*S. chouei* type)

⊠ *S. s. sadonis* in Sado   ◈ *S. s. shikokensis* in Shikoku

● *S. caecutiens* in Hokkaido
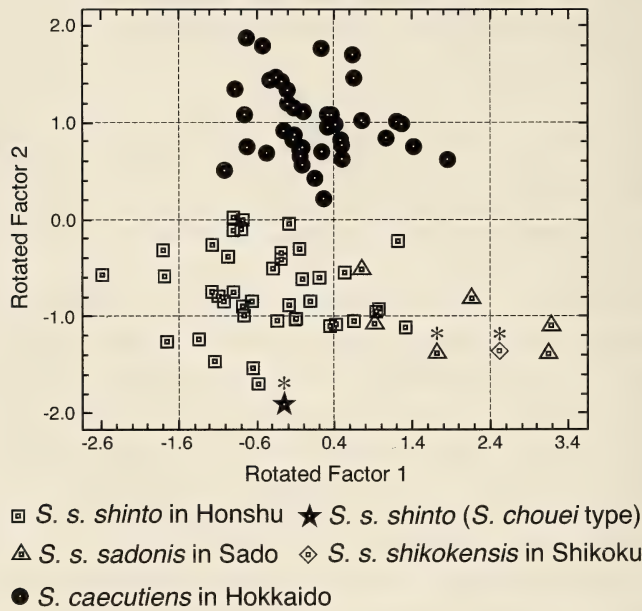
Fig. 2.   Plot of the first two factor scores for nine cranial and dental characters of shrews of the *Sorex caecutiens/shinto* group on the Japanese Islands.   Three symbols with asterisks (*) are the holotypes for *S. chouei*, *S. c. shikokensis*, and *S. sadonis*, which are treated as *S. s. shinto*, *S. s. shikokensis*, and *S. s. sadonis* in the present study, respectively.

Table 3. Varimax rotated factor matrix for nine cranial and dental characters of the *Sorex caecutiens/shinto* group in Japan. See the caption of Fig. 1 for abbreviation.

Character	Rotated loadings	
	I	II
Width across M^2	0.931	0.089
Width across U^2	0.861	0.154
Length of upper molariform tooth row	0.833	0.356
Condylobasal length	0.686	0.573
Facial length	0.667	0.507
Glenoid width	0.665	0.473
Cranial breadth	0.587	0.678
Relative width of Pm^4 mesostyle	0.063	0.922
Length of upper unicuspid row	0.333	0.899
Percent of total variance explained	45.8%	34.3%
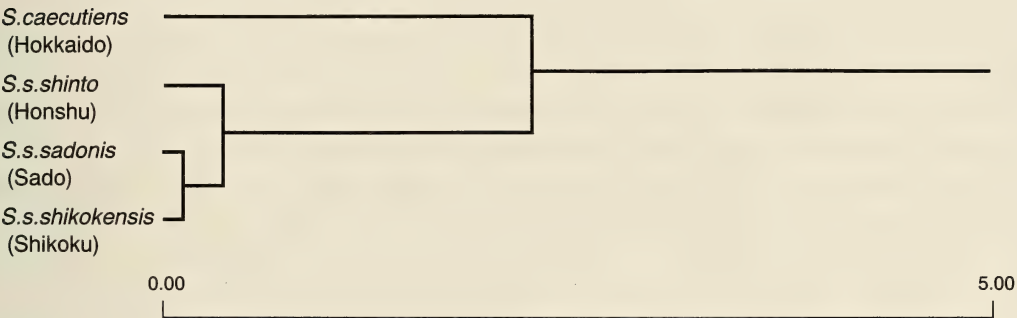
Fig. 3. A dendrogram generated by cluster analysis of nine cranial and dental characters of shrews of the *Sorex caecutiens/shinto* group on the Japanese Islands, based on single linkage method. The distance is multivariate Euclidean distance of the nine characters.

$$Z = -47.6 - 1.1(FL) + 8.9(LUU) + 4.9(U^2U^2) - 2.4(M^2M^2) + 0.6(RMW),$$

where FL=facial length, LUU=length of upper unicuspid row, U^{2}U^2=width across the second upper unicuspids, M^{2}M^2=width across the second upper molars, RMW=relative mesostyle width of Pm^4. The group centroids are +3.36 for the shrews of Hokkaido, and −2.58 for those of the Honshu complex. All the specimens were correctly classified into the two groups (probability of misclassification=0.0%).

Discussion

The morphometric analyses clearly showed that the shrews of Hokkaido (*S. caecutiens*) is morphologically different from the shrews of Honshu complex (*S. shinto* sspp.) (Figs. 2 and 3). The most important difference is in the shape of the upper premolar (Fig. 1 and Table 3). Dokuchaev (1978) found that *S. caecutiens* retains a well developed mesostyle of

Pm^4, which is a notable difference between it and several other shrew species.   This feature is consistent in *S. caecutiens* throughout its trans-continental Eurasian range.   In the present study, we found the same morphotype of the Pm^4 mesostyle in all of the Hokkaido shrews we examined (Fig. 1-B), while the mesostyle of Pm^4 of the shrews from the Honshu complex was less developed (Fig. 1-A).   For instance, the relative width of Pm^4 in Honshu shrews never reaches values found in Hokkaido shrews (Table 1).

George (1988) treated *S. shinto* from Honshu as a separate species from *S. caecutiens*, based on allozyme analysis.   Ohdachi et al. (1997a) showed that the shrews of Honshu and Shikoku were clearly distinct from those of Hokkaido, Sakhalin, and the Eurasian Continent, based on mitochondrial DNA sequences (see also Fumagalli et al. 1999).   According to their phylogenetical relationships (George 1988; Ohdachi et al. 1997a; Fumagalli et al. 1999) and their morphological differences (Figs. 2 and 3), *S. caecutiens* and *S. shinto* should be treated as two separate species.

In contrast, among the four OTUs from the Honshu complex (*S. s. shinto* including *S. chouei* holotype, *S. s. shikokensis*, and *S. s. sadonis*), no clear morphological demarcations were found, although only a small number of specimens were examined for the last three units (Fig. 2).   *Sorex chouei* was described on the basis of one specimen of an old individual with very worn teeth (Imaizumi 1954), and its holotype lay in an extreme point within the variation of *S. shinto* (Fig. 2), which might be attributed to by the very worn condition of its teeth.   *Sorex s. shikokensis* is a larger relative of *S. s. shinto* in Honshu (Abe 1967), however the genetic distance between them is very small (Ohdachi et al. 1997a).   The specimen of *S. s. shikokensis* dropped within the range of *S. s. sadonis* (Fig. 2) and was morphologically similar to the latter (Fig. 3).   In addition, we examined more than ten *S. s. shikokensis* that had over-wintered and confirmed that they were morphologically similar to *S. s. sadonis* (this data was not used in the present analyses in order to minimize the potential influence of age).

The molecular phylogenetical study suggested that the Sado Shrew, *S. s. sadonis*, should be considered as a subspecies or local population of *S. shinto* (Ohdachi et al. 1997a). Cranial and dental morphology confirmed that the Sado shrew was similar to the other taxa in the *S. shinto* complex (Fig. 3), and that there was morphological overlap between them (Fig. 2), although the Sado Shrews do have larger skulls than those of Honshu (as does *S. s. shikokensis*), longer claws on the forelegs, and darker pelage (Yoshiyuki and Imaizumi 1986).

According to Ohshima (1990, 1991, 1992), Sado Island was separated from proto-Honshu in the middle Pleistocene, long before the formation of the Tsugaru Strait, that separates Honshu and Hokkaido, which is estimated to have been formed 100–150 10^3-years ago.   In contrast, Ohdachi et al. (1997b) have doubted the earlier formation of the Sado Strait than the Tsugaru Strait, because of the molecular phylogeny of the *caecutiens/shinto* group.   Likewise, a more recent date for the isolation of Sado Island has been suggested by Tokuda (1941, 1969) on the basis of an examination of the distribution and morphological variation among rodents.   The Sado shrew might, therefore, have separated from the Honshu population of *S. shinto* recently (after 150 10^3-years ago at the most).   Furthermore, other small mammals, such as *Apodemus argenteus* (Temminck, 1844), *A. speciosus* (Temminck, 1844), and *Mogera tokudae* Kuroda, 1940 (the Sado mole) are found on both Sado and Honshu Islands (Abe 1994, 1995, 1997).   Fossil *A. argenteus* have been found from earlier periods in the Pleistocene than the genus *Sorex* from Honshu Island, and the earliest fossils of *A. speciosus* and *Mogera* sp. were from the same period as *Sorex*

sp. (Kawamura et al. 1989). In the case of *M. tokudae*, morphological and molecular phylogenetical characteristics of the populations of Sado and Honshu reveal that they are closely related to one other (Abe 1995; Okamoto 1998), as is the case in the shrews of the *caecutiens/shinto* group. At least, the extant *Apodemus* spp. and *M. tokudae* of Sado, whose origins seem to be older than or contemporaneous with *Sorex*, are considered conspecific with their Honshu counterpart populations. Therefore, the subspecific rank of *S. shinto sadonis* is considered to be the more appropriate taxonomic status for the Sado shrew, than *S. sadonis*, as suggested by Ohdachi et al. (1997a) and Koyasu (1998). In order to determine morphological status of *S. s. sadonis* within *S. shinto* more clearly, however, morphological comparisons, such as those of fur colour and claw length, should be conducted using larger sample sizes.

To summarize, morphological analysis has clearly demonstrated that *S. caecutiens* and *S. shinto* should be treated as separate species, as has previously been proposed by Ohdachi et al. (1997a) on the basis of their molecular phylogenetical study. Furthermore, morphological research also suggests that the shrew of Sado Island should be included within *S. shinto*.

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Appendix.

Codes of specimens used for analyses. Deposit places are National Science Museum (NSMT), Natural History Museum, Hokkaido University (NHMHU, HA, KM), and Institute of Low Temperature Science, Hokkaido University (SO).

Cranial Measures

S. caecutiens in Hokkaido

HA-1037, HA-1044, HA-1064, HA-1084, HA-1108, HA-1151, HA-1178, HA-1181, HA-1187, HA-1199, SO-88n105, SO-88n141, SO-88n169, SO-88n197, SO-88n203, SO-88n207, SO-88n248, SO-88n263, SO-88n264, SO-88n265, SO-88n285, SO-88n329, SO-88n336, SO-88n370, SO-88n377, SO-88n378, SO-89nn38, SO-96misc-5, SO-96misc-25, SO-96misc-26, SO-96misc-27, SO-96misc-28, SO-96misc-29, SO-96misc-30, SO-96misc-31, SO-96misc-32, SO-96misc-33, SO-96misc-34, SO-97/8/16-10, SO-97/9/1-1

S. s. shinto in Honshu

HA-1215, HA-6137, NSMT-M12479, NSMT-M12513 (holotype of *S. chouei* Imaizumi, 1954), NSMT-M13366, NSMT-M13397, NSMT-M13398, NSMT-M15593, NSMT-M15594, NSMT-M15595, NSMT-M15598, NSMT-M15599, NSMT-M15611, NSMT-M15613, NSMT-M16082, SO-95misc-2, SO-96misc-9, SO-96misc-10, SO-96misc-11, SO-96misc-13, SO-96misc-14, SO-96misc-15, SO-96misc-16, SO-96misc-17, SO-96misc-18, SO-96misc-19, SO-96misc-20, SO-96misc-21, SO-96misc-22, SO-96misc-57, SO-97/8/2-1, SO-97/8/5-1, SO-97/8/6-2, SO-97/8/6-3, SO-97/8/6-4, SO-97/8/6-5, SO-97misc-17, SO-97misc-18, SO-97misc-19, SO-97misc-37, SO-97misc-39, SO-97misc-40, SO-97misc-42, SO-97misc-134, SO-98misc-1

S. s. sadonis in Sado

NSMT-M16180 (holotype of *S. sadonis* Yoshiyuki et Imaizumi, 1986), NSMT-M26593, NSMT-M26600, NSMT-M26601, NSMT-M26602, NSMT-M27286

S. s. shikokensis in Shikoku

NHMHU-13311 (holotype of *S. caecutiens shikokensis* Abe, 1967)

External Measures

S. caecutiens in Hokkaido

KM-ki112, KM-ko103, KM-ko104, KM-ko121, KM-ko122, KM-ko50, KM-ko51, KM-ko52, KM-ko70, KM-

ko72, KM-ko74, KM-ko83, KM-ko92, KM-ko93, KM-ko94, KM-sh9, KM-tom1, KM-tom3, KM-tom4, KM-tom5, KM-tom7, KM-tom8, KM-tom9, KM-tom19, KM-tom20, KM-tom21, KM-tom22, KM-tom27, KM-tom28, KM-tom29, KM-tom30, KM-tom31, KM-tom32, KM-tom8-53, KM-tom8-54, KM-tom8-57, KM-tom8-58, KM-tom8-67, KM-tom8-68, KM-tom8-69, KM-tom8-70, KM-tom8-71, KM-tom8-72, KM-tom8-73, KM-tom8-74, KM-tom8-75, KM-tom8-76, KM-tom8-77, KM-tom8-78, KM-tom8-79, KM-tom8-82, KM-tom8-92, KM-tom8-93, KM-tom8-94, KM-tom8-95, KM-tom8-128, KM-tom8-130, KM-tom8-131, KM-tom8-132, KM-tom8-132/2, KM-tom8-133, KM-tom8-136, KM-tom8-139, KM-tom8-140, KM-tom8-143, KM-tom8-144, KM-tom8-145, KM-tom8-150, KM-tom8-151, KM-tom8-152, KM-tom8-153, KM-tom8-154, KM-tom8-156, KM-tom8-157, KM-tom8-163, KM-tom8-164, KM-tom8-165, KM-tom8-167, KM-tom8-168, KM-tom8-169, KM-tom8-171, KM-tom8-172, KM-tom8-176, KM-tom8-177, KM-tom8-178, KM-tom8-180, KM-tom8-181, KM-tom8-183, KM-tom9-31, KM-tom9-32, KM-tom9-34, KM-tom9-35, KM-tom9-36, KM-tom9-37, KM-tom9-38, KM-tom9-47, KM-tom9-48, KM-tom9-49, KM-tom9-51, KM-tom9-52, KM-tom9-58, KM-tom9-60, KM-tom9-71, KM-tom9-72, KM-tom9-73, KM-tom9-74, KM-tom9-75, KM-tom9-76, KM-tom9-77, KM-tom9-81, KM-tom9-82, KM-tom9-84, KM-tom9-86, KM-tom9-87, KM-tom9-93, KM-tom9-96, KM-tom9-97, KM-tom9-98, KM-tom9-130, KM-tom9-134, KM-tom9-137, KM-tom9-139, KM-tom9-140, KM-tom9-142, KM-tom9-144, KM-tom9-148, KM-tom9-149, KM-tom9-150, KM-tom9-151, KM-tom9-152, KM-tom9-153, KM-tom9-154, KM-tom9-157, KM-tom9-158, KM-tom9-159, KM-ton66a, SO-1-4, SO-30-1, SO-30-2, SO-31-1, SO-31-2, SO-88c025, SO-88c026, SO-88c060, SO-88c067, SO-88c068, SO-88f053, SO-88f065, SO-88f070, SO-88f096, SO-88f105, SO-88f110, SO-88f123, SO-88f126, SO-88f128, SO-88f132, SO-88f133, SO-88n105, SO-88n168, SO-88n169, SO-88n197, SO-88n203, SO-88n207, SO-88n248, SO-88n263, SO-88n264, SO-88n265, SO-88n274, SO-88n285, SO-88n336, SO-88t002, SO-88t006, SO-88t009, SO-88t012, SO-88t016, SO-89nn021, SO-89nn022, SO-89nn038, SO-89nn045, SO-94/9/13-8, SO-94/9/13-9, SO-94/9/13-10, SO-94/9/13-11, SO-94/9/14-7, SO-94/9/14-8, SO-94/9/14-9, SO-94/9/14-10, SO-94sc3, SO-95/7/12-3, SO-95/7/13-4, SO-95/7/13-10, SO-97/8/16-4, SO-97/8/16-5, SO-97/8/16-6, SO-97/8/16-7, SO-97/8/16-8, SO-97/8/16-9, SO-97/8/16-10, SO-97/8/16-11, SO-97/8/16-12, SO-97/8/16-13, SO-97/8/16-14, SO-97/8/16-15, SO-97/8/16-16, SO-97/8/16-17, SO-97/8/16-18, SO-97/8/17-5, SO-97/8/17-6, SO-97/8/17-7, SO-97/8/17-8, SO-97/8/17-9, SO-97/8/17-10, SO-97/8/17-11, SO-97/8/17-12, SO-97/8/17-13, SO-97/8/17-14, SO-97/8/17-15, SO-97/8/30-2, SO-97/8/31-1, SO-97/8/31-2, SO-97/8/31-9, SO-97/9/1-1, SO-97/9/19-8, SO-97/9/19-9, SO-97/9/19-10, SO-97/9/19-11, SO-98/6/19-6, SO-98/6/19-7, SO-98/6/20-10, SO-98/6/20-11, SO-98/6/20-12, SO-98/7/29-4, SO-98/7/29-5, SO-98/7/29-6, SO-98/7/30-6, SO-98/7/30-7, SO-98/7/30-8, SO-98/7/31-4, SO-98/7/31-5, SO-98/7/31-7

S. s. shinto in Honshu

SO-96misc-9, SO-96misc-10, SO-96misc-11, SO-96misc-13, SO-96misc-14, SO-96misc-15, SO-96misc-16, SO-96misc-17, SO-96misc-18, SO-96misc-19, SO-96misc-20, SO-96misc-21, SO-96misc-22, SO-97misc-37, SO-97misc-39, SO-97misc-40, SO-97misc-133, SO-97misc-134, SO-97/8/2-1, SO-97/8/5-1, SO-97/8/6-1, SO-97/8/6-2, SO-97/8/6-3, SO-97/8/6-4, SO-97/8/6-5

Constraints on feeding type in ruminants: a case for morphology over phylogeny

Jiang Zhaowen[1] and Seiki Takatsuki[2]

[1]*The Laboratory of Wildlife Biology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan*
[2]*The University Museum, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8654, Japan*

Abstract.   Ruminants were categorized into three feeding types: grazers, mixed feeders and browsers based on their food habits.   We studied how phylogeny constrains the feeding types, the morphology of digestive organs, and their relationships in Cervidae and Bovidae. It is shown that species with different feeding types occur in the same phylogenetic group of the family, subfamily, and tribe.   This suggests that phylogeny does not always reflect feeding type.   Comparisons of three morphological indices of digestive organs (parotid gland size, rumino-reticulum capacity, and rumino-reticulum contents weight) among feeding types found that trends along the grazer-browser continuum were similar in both families.   The index values of the same feeding types were similar in the two families.   These results suggest that the morphology of digestive organs is closely related to feeding types, and that phylogenetic characteristics are less important.   The species in the same feeding type also share other morphological characteristics of digestive organs, irrespective of phylogeny.

Key words: digestive organs, feeding type, morphology, phylogenetic constraint, ruminants.

The evolution of ruminants reflects changes in food quality and availability associated with changes in climate and vegetation during the Tertiary period (Romer 1966; Janis 1976). Adaptations were apparent in food habits, feeding behavior, morphology, and physiology of the digestive systems (Janis 1976; Hofmann 1989).

Studies on the feeding ecology of ungulates have revealed negative relationships between body mass and food quality (Bell 1971; Jarman 1974).   These authors emphasized the importance of body weight for the evolution of ungulate feeding ecology.   On the other hand, based on the comparative morphophysiology of digestive systems of 65 ruminant species (Hofmann 1968, 1989; Hofmann and Stewart 1972), Hofmann described the correspondence of feeding types and the morphology of digestive organs.   Considering the evolution of ruminants, he concluded that changes in feeding ecology and diets were the primary adaptive factors in ruminant evolution while body weight was secondary (Hofmann 1989).

Hofmann (1989) has categorized ruminants into three feeding types.   Of approximately 150 ruminants including six domestic species, about 25% fall into "grazers" which eat fibrous

foods rich in cell wall, or structural carbohydrates. For example, cattle, sheep, water buffalo, *Bubalus* spp., and banteng, *Bos javanicus*, belong to this group.

More than 40% of ruminant species belong to "browsers". They thrive on high quality diets and are adapted to process forage that is rich in plant cell contents. For example, roe deer, *Capreolus capreolus*, moose, *Alces alces*, and dik-dik, *Madoqua kirki*, are representative browsers.

The other 35% of ruminant species are "mixed feeders" which have intermediate characteristics between grazers and browsers. They show short term or seasonal changes in anatomy in response to food quality. The domestic goat, *Capra hircus*, and red deer, *Cervus elaphus*, belong to this group.

Most ruminologists and animal ecologists gave little attention to phylogenetic relationships in comparative studies of ruminant feeding ecology. However, the importance of considering phylogeny in comparative biology has been stressed thoroughly by Harvey and Pagel (1991). One of the most important arguments is that similar characteristics shown by different species do not necessarily imply adaptation to a particular environment, because it is possible that they have resulted merely from the phylogenetic history. However, if similar correlation between feeding types and morphological characteristics of digestive organs are observed in several independently evolving lineages, this implies that the traits have evolved in a correlated fashion, and explanations associated with the phylogenetic history are less likely to apply (Harvey and Pagel 1991).

The objective of this study is to clarify the importance of phylogenetic constraints on the feeding types and the morphology of digestive organs of ruminants. We classify species of Bovidae and Cervidae according to their feeding types. We then compare parameters of the morphology of digestive organs among the feeding types in each family, and the same feeding types between the two families.

Materials and methods

Groups examined and their feeding types

Species-level information for the three ruminant feeding types (grazers, mixed feeders, and browsers) was collected from available literature (references in Tables 1 and 2). We limited our search to two families, Bovidae (115 spp) and Cervidae (53 spp), because they account for 91.3% of the true ruminants (184 spp), and quantitative information is more available for these two families.

Taxonomic relationships of the species were used instead of phylogenetic relationships because the phylogenetic tree for all ruminant species is not available. The standard taxonomies of Spinage (1986) for Bovidae and Walker (1975) for Cervidae were adopted. For the classification of feeding types according to food habits, the results of Kay et al. (1980), Kay (1987), and Hofmann (1973, 1982, 1984) were used. Species not included in these studies were categorized according to food habit studies, i.e., the oryx, *Oryx basia* (Maloiy et al. 1982), bighorn sheep, *Ovis canadensis* (Belovsky 1986), Pere-David's deer, *Elaphurus davidianus* (Axmacher and Hofmann 1988), and Roosevelt elk, *Cervus elaphus roosevelti* (Church and Hines 1978). Based on feeding types, the taxonomic classifications of the ruminant species were rearranged.

Indices of digestive organs

Quantitative data on digestive organs of ruminants with different feeding types were collected from available literature (Tables 1 and 2). Three quantitative indices were derived: 1) ratio of parotid weight (g) to body weight (kg) (6 species of Cervidae and 14 species of Bovidae); 2) ratio of rumino-reticulum capacity (l) to body weight ($kg^{0.75}$) (6 species of Cervidae and 30 species of Bovidae); and 3) ratio of weight of rumino-reticulum contents to body weight ($kg^{0.75}$) (11 species of Cervidae and 36 species of Bovidae). Indices with inadequate sample size or otherwise unsuitable for quantitative comparison were used to describe qualitative differences between grazers and browsers (Table 3).

Data related to feeding type was available for 50 species and subspecies of Bovidae and 15 species and subspecies of Cervidae, including domestic species. For these subspecies and species, we could obtain data related to the three indices for 43 species and subspecies of Bovidae and 13 species and subspecies of Cervidae. Index values are expressed as mean $\pm SD$.

Results

Feeding types

Tables 1 and 2 show that both Bovidae and Cervidae include species belonging to different feeding types. Different feeding types were found not only in the two families, but also in lower taxonomic levels. In Bovidae, for example, the buffalo, *Syncerus caffer*, European bison, *Bison bonasus*, and greater kudu, *Tragelaphus strepsiceros*, in the sub-family Bovinae belonged to the grazers, mixed feeders, and browsers, respectively. Different feeding types were also found in subfamilies Antilopinae and Caprinae, and also at tribe levels, such as in Bovini, Antilopini, Neotragini, and Caprini. In Cervidae also, the sub-family Cervinae and the tribe Cervini included grazers and mixed feeders, and the sub-family Odocoilinae included both mixed feeders and browsers. There were, however, fewer species classified as grazers in Cervidae than in Bovidae.

Morphology of digestive organs

a. Parotid gland

In Bovidae, the relative weights of parotid glands were lower in grazers than those of mixed feeders and browsers, but no obvious difference was found between mixed feeders and browsers (Fig. 1, Table 1). In Cervidae, data were not available for grazers. The mean value for browsers was higher than that for mixed feeders, but variation was great (Fig. 1, Table 2).

Index values for each feeding type were similar in Bovidae and Cervidae. Index values showed a similar increasing tendency from grazers to browsers in both Bovidae and Cervidae (Fig. 1).

b. Rumino-reticulum capacity

In Bovidae, the relative capacities of rumino-reticula were greater in grazers than in mixed feeders and browsers (Fig. 1, Table 1). The mean value for mixed feeders was greater than that for browsers, but variation was great. Although only one datum was available for Cervidae browsers and grazers, respectively, relative capacity was larger in the grazer (Fig. 1,

Table 1. Parameters of digestive organs of species belonging to different feeding types in Bovidae.

Species		Body weight		Parotid	R-R capacity	R-R contents	Reference
English name	Scientific name	kg	$kg^{0.75}$	g/kg	$\% l/kg^{0.75}$	$\% kg/kg^{0.75}$	No.
Grazers							
Subfamily Bovinae							
Tribe Bovini							
Buffalo	*Syncerus caffer*	807.5	151.5		88.4	85.3	3, 4, 6
American bison	*Bison bison*	800.0	150.4			69.3	6, 12
European ox	*Bos taurus*	600.0	121.2	0.6		69.8	6, 13
Zebu	*Bos indicus*	400.0	89.4			63.3	6
Cow	*Bos taurus*	400.0	89.4		136.7	61.5	1, 2
Subfamily Reduncimae							
Mountain reedbuck	*Redunca fulvorufula*	23.5	10.7		68.4		3
Bohor reedbuck	*Redunca redunca*	45.0	17.4		55.8	20.1	3, 6
Laikipia waterbuck	*Kobus ellipsiprymnus*	220.0	57.1		76.9	52.5	3, 6, 9
Waterbuck	*Kobus defassa*	229.0	58.9			51.9	8
Nile lechwe	*Kobus megaceros*	80.0	26.7				6
Uganda kob	*Kobus kob*	79.0	26.5		36.8		3
Subfamily Hippotraginae							
Roan antelope	*Hippotragus equinus*	250.0	62.9				6
Sable antelope	*Hippotragus niger*	200.0	53.2				6
Oryx	*Oryx basia*	174.3	48.0			48.9	8
Oryx (Wild)	*Oryx gazella*	181.5	49.5		71.8	49.1	3, 4, 6
Oryx (Domesticated)	*Oryx gazella*	200.0	53.2			42.9	6
Subfamily Alcelaphinae							
Blue wildebeest	*Connochaetes taurinus*	182.0	49.6		80.7	57.3	3, 4, 5, 6
Hartebeest	*Alcelaphus buselaphus*	156.0	44.1		70.3	38.5	3, 5, 6
Topi	*Damaliscus lunatus*	119.0	36.0		86.0	44.2	3, 5, 6
Subfamily Antilopinae							
Tribe Antilopini							
Black buck	*Antilope cervicapra*	40.9	16.2	0.7			13
Tribe Neotragini							
Oribi	*Ourebia ourebi*	16.0	8.0		49.4		3
Subfamily Caprinae							
Tribe Caprini							
Ibex	*Capra ibex*	36.0	12.8				11
European sheep	*Ovis aries*	50.0	18.8	0.5	64.8	37.1	2, 5, 6, 13
Mouflon	*Ovis ammon musimon*	33.5	13.9	0.7	68.2	34.2	6, 11, 13
Mean ± SD				0.6 ± 0.1	73.4 ± 23.8	51.6 ± 16.1	
Mixed Feeders							
Subfamily Bovinae							
Tribe Bovini							
European bison	*Bison bonasus*	800.0	150.4			44.1	6
Tribe Tragelaphini							
Eland (Wild)	*Taurotragus oryx*	700.0	136.1		52.9	57.6	3, 6
Eland antelope (Pofu)	*Taurotragus oryx*	519.0	108.7		57.0	51.0	3, 7
Subfamily Aepycerotinar							
Impala	*Aepyceros melampus*	62.6	22.3		53.5	29.7	3, 4, 5, 6

Table 1. (continued)

Species		Body weight		Parotid	R-R capacity	R-R contents	Reference
English name	Scientific name	kg	kg$^{0.75}$	g/kg	% l/kg$^{0.75}$	% kg/kg$^{0.75}$	No.
Subfamily Antilopinae							
Tribe Antilopini							
Grant's gazelle	*Gazella granti*	64.0	22.6	2.0	56.6	23.9	3, 5, 6, 13
Thomson's gazelle	*Gazella thomsoni*	22.5	10.3	1.0	56.1	26.0	3, 5, 6, 13
Springbok	*Antidorcas marsupialis*	42.0	16.5	1.4	28.4	23.2	4, 6, 15
Tribe Neotragini							
Steinbok	*Raphicerus campestris*	10.5	5.8	2.2	42.9	13.5	3, 5, 6, 13
Subfamily Caprinae							
Tribe Ovibovini							
Musk ox	*Ovibos moschatus*	350.0	80.9				6
Tribe Rupicaprini							
Chamois	*Rupicapra rupicapra*	33.5	13.9	1.8	53.1		11
Tribe Caprini							
Goat	*Capra hircus*	40.0	15.9			44.6	5, 6
Dall's sheep	*Ovis dalli*	80.0	26.7				6
Bighorn sheep	*Ovis canadensis*	72.0	24.7			18.8	12, 14
Sheep	*Ovis aries*	30.0	12.8			44.9	6
Mean ± SD				1.7±0.5	50.1±9.8	34.3±13.9	
Browsers							
Subfamily Bovinae							
Tribe Tragelaphini							
Greater kudu	*Tragelaphus strepsiceros*	250.0	62.9			36.9	4, 6
Lesser kudu	*Tragelaphus imberbis*	90.5	29.3		45.0		3
Bushbuck	*Tragelaphus scriptus*	60.0	21.6		37.4	18.1	3, 5, 6
Bongo	*Taurotragus eurycerus*	200.0	53.2				6
Subfamily Cephalophinae							
Red duiker	*Cephalophus harveyi*	16.0	8.0	2.2	62.5	29.6	3, 6
Grey duiker	*Sylvicapra grimmia*	14.3	7.4		43.5	19.2	3, 5, 6
Subfamily Antilopinae							
Tribe Antilopini							
Gerenuk	*Litocranius walleri*	40.0	15.9	2.0	39.3	22.7	3, 6, 8, 13
Tribe Neotragini							
Klipspringer	*Oreotragus oreotragus*	12.0	6.4		26.1	12.1	6
Dik-dik	*Madoqua* spp.	5.2	3.4	1.6		10.2	3, 5, 6, 13
Gunther's dik-dik	*Madoqua quentheri*	4.1	2.9		26.0		3
Kirk's dik-dik	*Madoqua kirki*	5.2	3.4	1.5	27.4	10.8	1, 6, 10, 13
Suni	*Nesotragus moschatus*	4.5	3.1	1.6	25.5	12.3	3, 5, 6, 10, 13
Mean ± SD				1.8±0.3	37.0±12.4	19.1±9.2	

R-R: Rumino-reticulum. Reference No. 1. Short et al. (1965), 2. Prins and Geelen (1971), 3. Hofmann (1973), 4. Giesecke and Van Gylswyk (1975), 5. Hoppe et al. (1977), 6. Kay et al. (1980), 7. Demment (1982), 8. Maloiy et al. (1982), 9. Clemens and Maloiy (1983), 10. Hoppe et al. (1983), 11. Hofmann (1984), 12. Belovsky (1986), 13. Kay (1987), 14. Gordon and Illius (1988), 15. Hofmann et al. (1995).

Table 2). Index values for each feeding type were similar in Bovidae and Cervidae.

Table 2. Parameters of digestive organs of species belonging to different feeding types in Cervidae, Tragulidae, Camelidae and Giraffidae.

Species		Body weight		Parotid	R-R capacity	R-R contents	Reference
English name	Scientific name	kg	kg$^{0.75}$	g/kg	% l/kg$^{0.75}$	% kg/kg$^{0.75}$	No.
Cervidae							
Grazers							
Cervinae							
Pere-David's deer	*Elaphurus davidianus*	190.5	51.3				14
Cervini							
Sika deer	*Cervus nippon*	61.2	21.9		56.7	48.6	10, 16
Mixed feeders							
Cervinae							
Cervini							
Wapiti	*Cervus canadensis*	318.0	75.3			28.1	9, 12
Wapiti (Elk)	*Cervus elaphus roosevelti*	272.5	67.1				8
Red deer	*Cervus elaphus*	150.0	42.9	0.6	71.8	40.1	4, 7, 9, 13, 18
Fallow deer	*Dama dama*	70.0	24.2		35.2	29.0	4, 7, 9
Odocoilinae							
Odocoileini							
Mule deer	*Odocoileus hemionus*	120.0	36.3		28.5	22.6	2, 3, 9
White-tailed deer	*Odocoileus virginianus*	66.6	23.3		32.0	25.3	1, 9, 13
Caribou	*Rangifer tarandus acticus*	120.0	36.3			32.6	6, 9
Reindeer, Norwegian	*Rangifer tarandus tarandus*	90.0	29.2	0.6		41.5	9, 13, 17
Reindeer, Svalbard	*Rangifer t. platyrhynchus*	71.0	24.5	1.6		38.9	9, 13, 17
Mean ± SD				0.9±0.6	41.9±20.1	32.3±7.2	
Browsers							
Odocoilinae							
Alcini							
Moose	*Alces alces*	400.0	89.4			43.4	9
Capreolini							
Roe deer	*Capreolus capreolus*	20.7	9.7	2.2	15.5	15.9	4, 7, 9, 13
Hydropotinae							
Chinese water deer	*Hydropotes inermis*	12.0	6.4	1.4			9, 13, 15
Muntiacinae							
Reeves' muntjac	*Muntiacus reevesi*	10.4	5.8	1.4			9, 13
Mean ± SD				1.7±0.5	15.5	29.7±19.4	
Other families:							
Mixed feeder							
Camelidae							
Arabian camel	*Camelus dromedarius*			0.5			13
Antilocapridae							
Pronghorn	*Antilocapra americana*	50.0	18.8			33.8	6, 12
Browsers							
Tragulidae							
Larger mousedeer	*Tragulus napu*	4.0	2.8				9
Lesser mousedeer	*Tragulus javanicus*	1.5	1.4				9
Giraffidae							
Giraffe	*Giraffa camelopardalis*	750.0	143.3		73.2	70.3	5, 11

R-R: Rumino-reticulum.   References No. 1. Short (1964), 2. Short et al. (1965), 3. Hakonson and Whicker (1971), 4. Prins and Geelen (1971), 5. Hofmann (1973), 6. Hobson et al. (1975), 7. Nagy and Regelin (1975), 8. Church and Hines (1978), 9. Kay et al. (1980), 10. Hofmann (1982), 11. Maloiy et al. (1982), 12. Belovsky (1986), 13. Kay (1987), 14. Axmacher and Hofmann (1988), 15. Hofmann et al. (1988), 16. Takatsuki (1986), 17. Staaland and White (1991), 18. Fraser (1996).
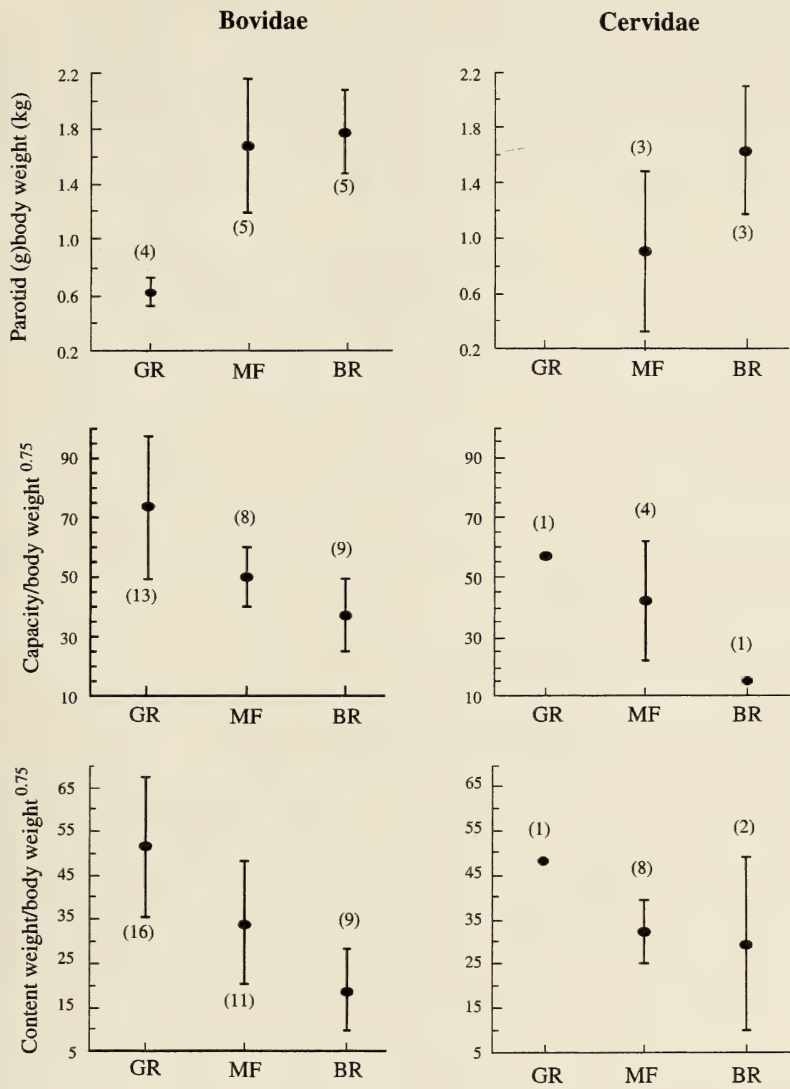
Fig. 1. Three indices of digestive organs of Bovidae and Cervidae. Top: weight contributions of parotid glands to body weight (g/kg), middle: ratios of capacity of rumino-reticulum (l) to metabolic body weight ($kg^{0.75}$), and down: ratio of content weight (kg) of rumino-reticulum to metabolic body weight ($kg^{0.75}$). GR: grazers, MF: mixed feeders and BR: browsers. Numbers in parentheses indicate sample size and vertical lines indicate *SD*. For data sources, see Tables 1 and 2.

c. Weight of rumino-reticulum contents

In Bovidae, the relative weight of rumino-reticulum contents was highest in grazers, followed by mixed feeders and lowest in browsers (Fig. 1, Table 1). A similar tendency was noted among the three feeding types in Cervidae, but only two data were available for browsers and one for grazers, respectively (Fig. 1, Table 2). Obvious difference was not found between mixed feeders and browsers (Fig. 1). Index values for each feeding type were similar in Bovidae and Cervidae.

Table 3. Characters of the digestive organs of grazers and browsers.

	Grazers	Browsers	Reference No.
Total salivary gland W (g)/Body W (kg)	1.8	3.6	5
Rumen structure	Subdivided	Simple	1
Rumen pillar	Powerful	Weak	1
Pappilla density in rumen wall	Low, uneven	High, even	2, 4
Rumen dorsal wall SEF	Low	High	2, 4
Average rumen SEF	Low	High	2, 4
Reticulum	Small, deeply cellulated	Large, lightly cellulated	1, 2
Omasum	Large, high SEF	Small, low SEF	1, 2
Orifice between reticulum and omasum	Small	Large	1, 2, 3
Abomasum	Large	Small	1, 2
Intestine L/Body L (time)	25–30	12–15	5
Small intestine L/Total intestine L (%)	80–82	65–73	5
Large intestine L/Total intestine L (%)	18–20	27–35	5
Capacity ratio of DFC/R-R	1/15–13	1/6–10	5

SEF: Surface enlargement factor of inner surface, L: Length, W: Weight, R-R: Rumino-reticulum. DFC: Distal fermentation chamber. Reference No. 1. Hofmann and Stewart (1972), 2. Hofmann (1973), 3. Kay et al. (1980), 4. Hofmann et al. (1988), 5. Hofmann (1989).

Other morphological characteristics of digestive organs

Other characteristics of digestive organs were compared for grazers and browsers (Table 3). The weight contribution of total salivary glands was higher in browsers than in grazers. The rumens of grazers were more subdivided, more capacious, and had more powerful muscle pillars for contraction than those of browsers (Hofmann 1968; Hofmann and Stewart 1972). Within the stomach, various shapes and sizes of papillae were more unevenly distributed in grazers than in browsers. Furthermore, papillae were less dense in grazers than in browsers (Hofmann 1968, 1973). In the dorsal regions of the stomachs of grazers, extensive unpapillated zones existed, while in browsers the papillae were evenly distributed. Consequently, the degree of rumen surface enlargement was smaller in grazers than in browsers (Hofmann 1973; Hofmann et al. 1988). The reticula of grazers were relatively smaller and more deeply cellulated than in browsers, and exhibited distinct subdivisions in the secondary and the tertiary crests (Hofmann 1973; Church and Hines 1978). The omasa were larger with more pronounced mucosal surface enlargement in grazers than in browsers, because of many laminae of several orders in size in grazers. The abomasa were larger and more spacious in grazers than in browsers. Orifices between reticula and omasa were smaller in grazers than in browsers (Hofmann 1973, 1989). Total intestinal length of grazers were relatively longer, but the large intestines were relatively shorter than those of browsers (Hofmann 1989). The distal fermentation chambers of grazers were relatively less spacious than those of browsers. The digestive systems of grazers can pass foods more slowly through the gut and digest them more thoroughly than those of browsers (Kay et al. 1980; Van Soest 1982; Hofmann 1989).

Discussion

In this analysis, species relationships were derived from the traditional taxonomic

classification of ruminants based mainly on morphology (Walker 1975; Spinage 1986). In spite of considerable advances in studies on the phylogenetic relationships of ruminants (Miyamoto et al. 1990; Gatesy et al. 1992; Wall et al. 1992; Chikuni et al. 1995; Cronin et al. 1996), a phylogenetic tree for all ruminants has not been established. However, phylogenetic studies have confirmed close relationships within genera as defined by traditional taxonomic categories. We assume that the use of traditional taxonomic relationships does not affect our results.

The indices analyzed are closely related to food habits and digestive physiology. The salivary gland and rumino-reticulum are the most important organs for ruminants to digest fibrous foods (Hofmann 1973). The amount of the saliva secreted by the parotid gland is closely related to the size of the glands, and the quick turnover and fermentation of ingesta in the stomach of browsers need more saliva to buffer the rumen's volatile fatty acid and pass the digesta (Kay 1987; Hofmann 1989). Therefore, browsers possess larger parotid glands than do grazers. In contrast to the salivary glands of the browsers, grazers possess a larger rumino-reticulum, which ferments and digests fibrous food efficiently by its long retention time (Hofmann 1973, 1989).

The result that different species in the same phylogenetic group (Bovidae or Cervidae) appear in different feeding types suggests that the feeding types are not constrained strongly by phylogenetic relations. The average values for each index showed a similar tendency from grazers to browsers in both Bovidae and Cervidae (Fig. 1). In addition, other morphological characteristics of the digestive organs of ruminants are similar in species of the same feeding type irrespective of the phylogenies (Table 3). This further suggests that the morphologies of digestive organs associated with different feeding types are likely consistent in different phylogenetic groups, and are not strongly constrained by phylogeny. These results suggest that the correlation among characteristics reflects independently evolving lineages in ruminants. It is, however, noteworthy that Cervidae contains fewer grazers, suggesting that species numbers are affected by phylogeny.

Two conclusions arise from this study: 1) phylogenetic relations did not strongly constrain feeding types and morphology of digestive organs of ruminants, and 2) there exists an underlying principle that feeding types of ruminants are closely related to the morphology of their digestive organs even in different phylogenetic groups.

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Diet of the Japanese serow (*Capricornis crispus*) on the Shimokita Peninsula, northern Japan, in reference to variations with a 16-year interval

Keiji Ochiai[1]

Natural History Museum and Institute, Chiba, 955-2 Aoba-cho, Chuo-ku, Chiba 260-8682, Japan

Abstract.   The diet of the Japanese serow (*Capricornis crispus*) was analyzed quantitatively in a high density (14.8±3.0 individuals/km²) population throughout the year by direct observation of feeding behavior on the Shimokita Peninsula, northern Japan, during two survey periods, 1978–1980 and 1994–1996.   Serows fed on 114 plants species and one species of fungus.   Analyses of 16,686 bites indicated that serows fed mainly on leaves and twigs of deciduous broad-leaved trees, which formed 54.8–58.3% of the diet in autumn and 94.5–95.0% in winter, followed by forbs (16.5–39.1% from spring to autumn).   The results suggest that the Japanese serow is a browser throughout the year, and is mainly a folivore. There was no significant difference in the dietary composition at the food category level, nor was there any change in the diversity index of the diet between the two study periods.   The four top-ranking food species were identical in the both periods.   Browsing by Japanese serows may have only limited impacts on vegetation because of low population densities related to territoriality.

Key words: browser, browsing effects, *Capricornis crispus*, diet, Japanese serow.

The Japanese serow (*Capricornis crispus*) is a solitary ungulate inhabiting forested mountainous areas of Japan.   A knowledge of food habits is essential for understanding wildlife habitat needs.   The present study on the diet of Japanese serows had two aims.

The first was to analyze the serow diet quantitatively throughout the year.   Many reports (Chiba 1968; Chiba and Yamaguchi 1975; Miyao 1976; Akasaka 1977; Akasaka and Maruyama 1977; Yamaguchi and Takahashi 1979) have indicated that Japanese serows feed mainly on various woody species according to the region inhabited.   However, these studies have been qualitative and/or analyzed with small sample size, except for a single analysis of the winter diet (Takatsuki and Suzuki 1984).   Therefore, more detailed quantitative studies on all seasons are needed for a clear understanding of Japanese serow feeding habits.

The second aim of the study was to analyze the effects of browsing by Japanese serows on vegetation by comparing the diets recorded in two survey periods 16 years apart. Ungulates not only depend on plant communities but also can affect plant community composition and structure.   Numerous studies on the effects of browsing have been conducted,

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[1]*E-mail: ochiai@chiba-muse.or.jp*

as reviewed by Gill (1992) and Augustine and McNaughton (1998).   However, almost all of these studies focused on gregarious and/or non-territorial ungulates.   The effects of solitary and territorial ungulates such as the Japanese serow on vegetation may be different from those of gregarious species.   In this study, the effects of serows on the vegetation are discussed in comparison with corresponding information on sika deer (*Cervus nippon*), a gregarious ungulate inhabiting Japan.

Study area

The study area (90 ha) was situated in Wakinosawa village (41°8′N; 140°46′E) on the Shimokita Peninsula, Aomori Prefecture, northern Japan.   The area, facing Mutsu Bay on its south and west sides, ranges in altitude between 0 and 240 m, and slopes are steep (25°). The climate belongs to the cool temperate zone; mean annual temperature is 9.3°C and mean monthly temperatures ranges from -1.8°C in February to 21.5°C in August.   Mean annual precipitation is 1,337 mm at the nearest meteorological station, 4 km east of the study area. The area is covered by 30–100 cm of snow in winter, and snow cover persists for three months between late December and March.

In 1978, 75% of the area was mature deciduous broad-leaved forests dominated by *Quercus mongolica* ssp. *crispula*, *Fagus crenata* and *Tilia japonica* with *Rhododendron obtusum* var. *kaempferi* and *Viburnum dilatatum* as common understory species.   Natural coniferous forests of *Thujopsis dolabrata* var. *hondae* covered 7% of the area.   Plantations with coniferous trees (*Cryptomeria japonica* and *Pinus densiflora*) both less than 20 years old and over 21 years old accounted for 10% and 3% of the area, respectively.   The vegetation composition in the study area remained unchanged between the two study periods.   The proportion of younger and older plantations, however, had changed to 1% and 12% of the study area.

Serows, the only species of ungulates inhabiting the study area, maintained a stable population density with a mean of 14.8 individuals/km^2 ($SD=3.0$) from 1976 to 1996 (Ochiai 1993, unpublished data).   The mean population density of Japanese serows in 10 prefectures is 2.6 ± 2.7 individuals/km^2 (mean $\pm SD$, $n=174$) (Maruyama and Furubayashi 1980); the density of serows in the study area was at the highest level.

Methods

To servey vegetation composition in the study area, trunk diameters at ground level of all woody plants (<2 m in height) were measured in forty 5 m $\times$ 4 m plots.   For the survey two sites in the study area were selected, and the twenty plots in each site were distributed uniformly on a 100 m square grid.   This survey was carried out in 1997.

Diet composition was estimated by direct bite count observation (Wallmo and Neff 1970).   Field observations were made during two periods, from March 1978 to October 1980 (Period A, 191 days) and from October 1994 to November 1996 (Period B, 59 days).   The surveys were conducted in May–June (spring), July–September (summer), October–November (autumn) and January–March (winter) in Period A and only in autumn and winter in Period B.   Serows were directly observed for 947 h in Period A and 223 h in Period B, for a total of 1,170 h in 250 days.

I walked through the study area looking for serows, and observed the feeding behavior of any serows detected with the aid of 7×35 binoculars and a telescope ($\times 25$). Because the serows in the study area were not very alarmed by the presence of humans, it was possible to follow about 10–20 m behind without serious disturbances. The number of bites, food species and plant parts eaten were recorded. In winter, fresh signs of feeding on twigs along fresh serow tracks in snow were also recorded as bites. Foods were categorized into woody plants (deciduous broad-leaved trees, including vines, and evergreen coniferous trees), forbs, graminoids, ferns and fungi. The composition of serow diets was expressed as percentages of the total number of bites, regardless of bite size.

Food diversity was measured according to the Shannon-Weaver index (H'),

$$H' = -\Sigma(P_i)\ln(P_i)$$

where P_i is the proportion of food item i in the total diet.

Diets in autumn and winter between Periods A and B were compared by the G-test.

Results

Dominance of understory trees

Forty-four understory tree species were recorded in the plots, and deciduous broad-leaved trees of 41 species accounted for as much as 96.6% of the total basal area at ground level (Appendix 1). Two species of deciduous broad-leaved trees, *R. obtusum* var. *kaempferi* (18.5%) and *V. dilatatum* (18.3%), were dominant, followed by *Q. mongolica* ssp. *crispula* (8.0%), *Tripetaleia paniculata* (6.5%) and *T. japonica* (5.4%).

Diet at the food category level

A total of 16,686 bites was observed. The most important food category was woody plants, which comprised 56.2–60.2% of the diet in autumn to 97.7–98.1% in winter (Table 1). Forbs accounted for 16.5–39.1% from spring to autumn, and two graminoids (*Carex* species) were eaten in autumn (3.9–4.7%) and winter (1.6–2.1%).

Among trees, deciduous broad-leaved trees were the primary food. Serows browsed leaves from spring to autumn and 5–10 cm long twigs with buds in winter. The proportion of the twigs rose to 93.2–94.5% in the winter diet. Leaves of evergreen coniferous trees were eaten in autumn (1.4–1.9%) and winter (2.7–3.6%). Serows occasionally fed on flowers and fruit together with leaves. Flowers of *R. obtusum* var. *kaempferi* and fruits of *Berberis amurensis* were eaten selectively. Fallen acorns of *Q. mongolica* ssp. *crispula* were also consumed in autumn and winter. Serows dug for acorns when the snow cover was not more than 10 cm deep. No bark or dead leaves of woody plants were eaten.

Diet at individual species level

Serows fed on 114 plant species (60 deciduous broad-leaved trees, 5 evergreen coniferous trees, 46 forbs, two graminoids and one fern) belonging to 56 families, and one species of fungus (Table 2). The numbers of food species were higher (64–81 species) from spring to summer, and lower (29–32 species) in winter. The diversity index (H') of the diet was highest (3.29) in summer, and lowest (2.33–2.35) in winter (Table 2).

Table 1. Food categories and the percentage frequencies of bites of the Japanese serow (*Capricornis crispus*) observed on the Shimokita Peninsula, northern Japan, in each season during Period A (1978–1980) and Period B (1994–1996).

Month	May–June	July–Sept.	Oct.–Nov.		Jan.–Mar.	
Period	A	A	A	B	A	B
Food category	n=2,281	n=3,567	n=2,668	n=1,720	n=3,879	n=2,571
Woody plants						
Deciduous broad-leaved trees						
leaves	82.9	77.8	56.1	49.1	0.0	0.0
flowers	0.6	0.0	0.0	0.0	0.0	0.0
fruits	0.0	0.6	0.2	0.3	0.0	0.0
acorns	0.0	0.0	0.4	5.4	0.0	1.8
twigs and buds	0.0	0.0	1.6	0.0	94.5	93.2
Total deciduous broad-leaved trees	83.5	78.4	58.3	54.8	94.5	95.0
Evergreen coniferous trees (leaves)	0.0	0.0	1.9	1.4	3.6	2.7
Total woody plants	83.5	78.4	60.2	56.2	98.1	97.7
Forbs						
leaves and stems	16.5	21.1	35.8	39.1	0.3	0.2
flowers	0.0	0.4	0.1	0.0	0.0	0.0
Total forbs	16.5	21.5	35.9	39.1	0.3	0.2
Graminoids	0.0	0.0	3.9	4.7	1.6	2.1
Ferns	0.0	0.0	+	0.0	0.0	0.0
Fungi	0.0	0.1	0.0	0.0	0.0	0.0

+: trail (<0.05).

Table 2. Number of food species for each food category, diversity index of diet, and percentage frequencies of bites accounted for by major food species (3, 5 and 10 top-ranking food species) of the Japanese serow (*Capricornis crispus*) on the Shimokita Peninsula, northern Japan, in each season during Period A (1978–1980) and Period B (1994–1996).

Food category	Period	May–June	July–Sept.	Oct.–Nov.		Jan.–Mar.		Total
		A	A	A	B	A	B	—
Woody plants								
Deciduous broad-leaved trees		39	42	28	23	23	24	60
Evergreen coniferous trees		0	0	2	1	5	2	5
Forbs		25	38	23	23	2	1	46
Graminoids		0	0	2	2	2	2	2
Ferms		0	0	1	0	0	0	1
Fungi		0	1	0	0	0	0	1
Total		64	81	56	49	32	29	115
Shannon-Wiener diversity index		2.87	3.29	3.03	3.09	2.33	2.35	—
% of the 3 top-ranking food species		50.7	35.7	39.4	39.4	59.0	57.8	—
% of the 5 top-ranking food species		59.1	46.4	53.9	53.2	73.7	75.5	—
% of the 10 top-ranking food species		74.4	64.0	73.0	71.0	88.6	88.6	—

In spite of the large number of food species, a major part of the diets comprised a limited number of species: e.g., the top five and 10 species in the diet accounted for 46.4–75.5% and 64.0–88.6%, respectively (Table 2). According to a similar analysis, 80% of the total diet was comprised of 13–18 species in spring-autumn and only seven species in winter. The main food species changed seasonally (Appendix 2).

Comparison between Period A (1978–1980) and Period B (1994–1996)

No significant difference in the percentage frequencies of bites of the four food categories (deciduous broad-leaved trees, evergreen coniferous trees, forbs and graminoids) was detected in either autumn or winter between the two study Periods A and B (Table 1; autumn, $G=7.65$, $df=3$, $P>0.05$; winter, $G=6.78$, $df=3$, $P>0.05$). There was no obvious change in the H' values for the diet between Periods A and B; the values were 3.03 and 3.09 in autumn and 2.33 and 2.35 in winter, respectively. The following species remained unchanged as the top four species in the both periods: *Callicarpa japonica*,

Table 3. Results of *G*-test for the numbers of counted bites of the Japanese serow (*Capricornis crispus*) on the Shimokita Peninsula, northern Japan, in autumn between Period A (1978–1980) and Period B (1994–1996). The top 15 species in each period are shown.

Species	Period A			Period B			G	
	Rank order	No. of bites	%	Rank order	No. of bites	%		
Callicarpa japonica	1	464	17.4	1	324	18.8	0.739	NS
Alangium platanifolium var. *trilobum*	2	345	12.9	4	136	7.9	14.052	D***
Angelica ursina	3	242	9.1	2	201	11.7	3.887	I*
Artemisia montana	4	237	8.9	3	152	8.8	0.001	NS
Buckleya lanceolata	5	150	5.6	—	0	0.0	76.335	D***
Akebia trifoliata	6	135	5.1	9	49	2.8	6.689	D**
Smilax china	7	124	4.6	11	35	2.0	11.041	D***
Carex blepharicarpa & *C. foliosissima*	8	105	3.9	6	82	4.8	0.877	NS
Asperula odorata	9	75	2.8	44	4	0.2	25.586	D***
Rhus ambigua	10	70	2.6	33	7	0.4	18.188	D***
Spuriopimpinella calycina	11	66	2.5	21	16	0.9	7.477	D**
Rubus crataegifolius	12	61	2.3	16	25	1.5	1.962	NS
Acer japonicum	13	54	2.0	14	28	1.6	0.455	NS
Eupatorium chinense var. *oppositifolium*	14	48	1.8	24	14	0.8	3.927	D*
Berberis amurensis	15	43	1.6	—	0	0.0	21.530	D***
Quercus mongolica ssp. *crispula*	19	35	1.3	5	114	6.6	44.492	I***
Cacalia auriculata var. *kamtschatica*	27	13	0.5	13	29	1.7	7.718	I**
Schizophragma hydrangeoides	41	3	0.1	10	45	2.6	32.747	I***
Solidago virgaurea var. *asiatica*	41	3	0.1	12	32	1.9	21.386	I***
Lindera umbellata ssp. *membranacea*	46	2	0.1	15	26	1.5	18.249	I***
Zanthoxylum piperitum	—	0	0.0	7	79	4.6	75.113	I***
Trifolium repens	—	0	0.0	8	52	3.0	49.184	I***
Others	—	393	14.7	—	270	15.7	—	—
Total	—	2,668	100.0	—	1,720	100.0	—	—

Asterisks indicate the degree of significance of the results (*** $P<0.001$, ** $P<0.01$, * $P<0.05$, NS=not significant), and 'D' and 'I' represent a significant decline and increase in occurrence of the individual species in the diet from Period A to B, respectively.

Table 4. Results of *G*-test for the numbers of counted bites of the Japanese serow (*Capricornis crispus*) on the Shimokita Peninsula, northern Japan, in winter between Period A (1978–1980) and Period B (1994–1996). The top 15 species in each period are shown.

Species	Period A			Period B			*G*	
	Rank order	No. of bites	%	Rank order	No. of bites	%		
Hamamelis japonica var. *obtusata*	1	1,112	28.7	2	572	22.2	16.727	D***
Lindera umbellata ssp. *membranacea*	2	858	22.1	1	583	22.7	0.138	NS
Tilia japonica	3	317	8.2	4	257	10.0	3.138	I*
Acer japonicum	4	292	7.5	3	332	12.9	25.103	I***
Viburnum furcatum	5	279	7.2	7	75	2.9	29.536	D***
Stachyurus praecox	6	211	5.4	15	11	0.4	75.640	D***
Thujopsis dolabrata var. *hondae*	7	120	3.1	8	71	2.8	0.299	NS
Carpinus cordata	8	97	2.5	11	55	2.1	0.444	NS
Viburnum wrightii	9	86	2.2	5	197	7.7	53.540	I***
Quercus mongolica ssp. *crispula*	10	64	1.6	6	111	4.3	20.296	I***
Fraxinus lanuginosa f. *serrata*	11	64	1.6	10	57	2.2	1.329	NS
Carex blepharicarpa & *C. foliosissima*	12	61	1.6	12	52	2.0	0.897	NS
Corylus sieboldiana	13	55	1.4	16	9	0.4	10.345	D**
Viburnum dilatatum	14	52	1.3	9	67	2.6	6.657	I**
Buckleya lanceolata	15	50	1.3	—	0	0.0	25.553	D***
Fagus crenata	19	18	0.5	14	38	1.5	9.020	I**
Rhus ambigua	24	9	0.2	13	42	1.6	19.595	I***
Others	—	134	3.5	—	42	1.6	—	—
Total	—	3,879	100.0	—	2,571	100.0	—	—

Asterisks indicate the degree of significance of the results (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = not significant), and 'D' and 'I' represent a significant decline and increase in occurrence of the individual species in the diet from Period A to B, respectively.

Alangium platanifolium var. *trilobum*, *Angelica ursina*, and *Artemisia montana* in autumn; *Hamamelis japonica* var. *obtusata*, *Lindera umbellata* ssp. *membranacea*, *Tilia japonica*, and *Acer japonicum* in winter. However, in the second study period, among 22 species in autumn (the top 15 species in each period) nine accounted for a significantly lower proportion of the diet, eight accounted for a significantly higher proportion ($P < 0.05$), and five showed no significant difference (Table 3). There were some conspicuous declines between study periods (e.g., *Buckleya lanceolata*, *Asperula odorata*). Among 17 species in winter, the proportion in the diet of five significantly decreased, seven increased ($P < 0.05$), and five showed no significant difference (Table 4). Conspicuous declines between study periods were also apparent in the winter use of some species (e.g., *Stachyurus praecox*, *Viburnum furcatum*).

Discussion

General features of the serow diet

On the Shimokita Peninsula, Japanese serows fed mainly on woody plants, mostly deciduous broad-leaved trees. Forbs were of secondary importance from spring to autumn. These results support the findings of Takatsuki and Suzuki (1984), who analyzed the winter

foods of serows in central Japan, and described them as browsers. The current study found that Japanese serows on the Shimokita Peninsula are browsers throughout the year, and are conspicuous folivores.

Food habits of Japanese serows vary regionally. Several pioneer reports indicated that conifers and evergreen broad-leaved shrubs are important in the winter diet (Chiba and Yamaguchi 1975; Miyao 1976; Akasaka 1977; Yamaguchi and Takahashi 1979). For instance, the proportion of coniferous trees occupied more in winter in central Japan (32.8%, Takatsuki and Suzuki 1984) than in the present study (2.7–3.6%). Their study areas contained many young plantations of a coniferous tree (*Chamaecyparis obtusa*). Takatsuki et al. (1988) reported that a coniferous shrub (*Cephalotaxus harringtonia* var. *nana*) and an evergreen broad-leaved shrub (*Aucuba japonica* var. *borealis*) were the main winter foods in Yamagata, northern Japan. These shrubs are uncommon in the present study area. In addition, no feeding on dwarf bamboos (*Sasa* spp.) was observed in the present study, although it accounted for 27.2% of the winter diet in central Japan (Takatsuki and Suzuki 1984). Takatsuki and Suzuki (1984) interpreted the importance of dwarf bamboos in the diet of serows as a reflection of their relative abundance and year-round availability. Dwarf bamboos seem to be less important in low altitude areas (<600 m) such as the present study and Yamagata (Takatsuki et al. 1988) than at higher altitudes (600–1700 m, Chiba and Yamaguchi 1975; Yamaguchi and Takahashi 1979; Takatsuki and Suzuki 1984). If this altitude-related difference is valid, dwarf bamboos may be a less preferred food eaten mainly under poor food conditions.

The Japanese serow is a forest-dwelling, solitary species with a small resource-defending territory (Akasaka and Maruyama 1977; Ochiai 1983a, b, 1993; Kishimoto and Kawamichi 1996). Their body size is moderate (30–45 kg), and they have little sexual dimorphism (Miura 1986). These features are typical of browsers (Bell 1971; Jarman 1974). The present results together with the findings of Takatsuki and Suzuki (1984) support the Jarman-Bell principle.

The mainland serow (*C. sumatraensis*) and the goral (*Nemorhaedus goral*) belong to the same Tribe Rupicaprini as the Japanese serow. These are also primitive species inhabiting forests. Faecal analysis of these two sympatric species in North India indicated that the mainland serow was a browser, whereas the goral was a grazer (Green 1987). The goral can dwell in cliff habitats with open rocky slopes (Schaller 1977; Heptner et al. 1989; Lovari and Apollonio 1993). The maximum group size of gorals is 9–11 (Cavallini 1992; Lovari and Apollonio 1993), which is larger than the corresponding figure, 4 of the Japanese serow (Ochiai 1993; Kishimoto and Kawamichi 1996). The Japanese serow seems to be a fairly solitary browser in comparison with the goral.

Effects of browsing

In the 16 years between study Period A and B, no obvious difference in the crude composition of the diet was found. However, the proportion of some species, such as *Buckleya lanceolata* and *Stachyurus praecox*, decreased markedly in the diet. These results suggest that although the browsing of serows may have no drastic impacts on overall vegetation structure and composition, some species may be affected by browsing pressure. Since the density of serows in the study area is among the highest in Japan, negative impacts of browsing on vegetation structure and composition in other areas are even less likely.

In contrast, the sika deer (*Cervus nippon centralis*), another medium-sized ruminant (60–87 kg for males and 40–50 kg for females, Koganezawa et al. 1986; Ochiai and Asada 1995) living in Honshu, Japan, has been reported to have severe impacts on vegetation, including not only the elimination of the main food species in the habitat (Kabaya 1988) but also the alteration of forest composition (Takatsuki and Gorai 1994). This difference may reflect differences in social organization, such as the territorial isolation of the Japanese serow as opposed to the non-territorial gregariousness of sika deer. Consequently, the population density of serows seldom exceeds 20/km^2 (Maruyama and Furubayashi 1980), whereas it reaches 100/km^2 with sika deer (*C. n. nippon*; Doi and Endo 1995). At such low densities, it is less likely that over-browsing by serows would lead to changes in overall vegetation structure and composition (Takatsuki 1996). Furthermore, the aggregation of serows and the concentration of their browsing on preferable species may be less than sika deer due to their territoriality, even when their population densities are similar. Differences in the volume of forage intake related to body size may also influence the relative degree of the browsing effects.

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Appendix 1. Basal area of understory woody species in the study area.

Species	Leaf type*	Basal area** (mm²/m²)	Percentage
Rhododendron obtusum var. *kaempferi*	D	63.3	18.5
Viburnum dilatatum	D	62.4	18.3
Quercus mongolica ssp. *crispula*	D	27.2	8.0
Tripetaleia paniculata	D	22.1	6.5
Tilia japonica	D	18.5	5.4
Lindera umbellata ssp. *membranacea*	D	16.3	4.8
Fraxinus lanuginosa f. *serrata*	D	15.1	4.4
Magnolia praecocissima	D	14.5	4.2
Hamamelis japonica var. *obtusata*	D	9.6	2.8
Viburnum wrightii	D	9.3	2.7
Acer japonicum	D	9.0	2.6
Callicarpa japonica	D	8.0	2.3
Carpinus cordata	D	7.8	2.3
Thujopsis dolabrata var. *hondae*	C	6.1	1.8
Zanthoxylum piperitum	D	5.5	1.6
Vaccinium oldhamii	D	5.4	1.6
Syringa reticulata	D	4.4	1.3
Evodiopanax innovans	D	4.2	1.2
Quercus dentata	D	3.7	1.1
Acer mono f. *marmoratum*	D	3.7	1.1
Alangium platanifolium var. *trilobum*	D	3.5	1.0
Rhododendron brachycarpum	E	3.0	0.9
Fagus crenata	D	3.0	0.9
Cryptomeria japonica	C	2.3	0.7
Corylus sieboldiana	D	2.3	0.7
Pourthiaea villosa var. *laevis*	D	1.9	0.5
Carpinus laxiflora	D	1.8	0.5
Rhus ambigua	D	1.7	0.5
Euonymus alatus f. *ciliato-dentatus*	D	1.0	0.3
Sorbus alnifolia	D	0.9	0.3
Viburnum furcatum	D	0.9	0.3
Benthamidia japonica	D	0.7	0.2
Berberis amurensis	D	0.7	0.2
Rubus crataegifolius	D	0.6	0.2
Aralia elata	D	0.2	0.1
Sambucus racemosa ssp. *sieboldiana*	D	0.2	0.1
Vaccinium japonicum	D	0.2	0.1
Clerodendrum trichotomum	D	0.2	0.1
Picrasma quassioides	D	0.2	0.0
Euonymus alatus	D	0.1	0.0
Morus australis	D	0.1	0.0
Buckleya lanceolata	D	0.1	0.0
Stachyurus praecox	D	+	0.0
Akebia trifoliata	D	+	0.0
Total	—	341.6	100.0

*: D, deciduous broad-leaved tree (including two vine species); E, evergreen broad-leaved tree; C, evergreen coniferous tree.

**: basal area at ground level.

+: trail (<0.05 mm²/m²).

Appendix 2. Food species and the percentage frequencies of observed bites of the Japanese serow (*Capricornis crispus*) on the Shimokita Peninsula, northern Japan, in each season Period A (1978–1980) and Period B (1994–1996). Sixty-one species of the entire serow diet (145 species), which are the top 20 species in each season, are shown in the present list.

Species	Family name	Life form*	Part eaten**	Total	Spring Period A	Summer Period A	Autumn Period A	Autumn Period B	Winter Period A	Winter Period B
Callicarpa japonica	Verbenaceae	D	L, T	8.7	0.1	16.3	17.4	18.8	0.6	0.1
Viburnum dilatatum	Caprifoliaceae	D	L, T	6.9	25.6	0.1			1.3	2.6
Hamamelis japonica var. *obtusata*	Hamamelidaceae	D	L, T	6.6	0.4		0.1		28.7	22.2
Lindera umbellata ssp. *membranacea*	Lauraceae	D	L, T	6.5	2.7	0.3	0.1	1.5	22.1	22.7
Tilia japonica	Tiliaceae	D	L, T	5.0	8.0	3.1	0.1		8.2	10.0
Buckleya lanceolata	Santalaceae	D	L, T	4.5	1.6	12.4	5.6		1.3	
Acer mono f. *marmoratum*	Aceraceae	D	L, T	4.5	17.1	0.9	0.2			0.2
Alangium platanifolium var. *trilobum*	Alangiaceae	D	L	4.5		7.0	12.9	7.9		
Acer japonicum	Aceraceae	D	L, T	4.4	3.8	2.1	2.0	1.6	7.5	12.9
Artemisia montana	Compositae	F	L, S, Fl	3.6	1.2	4.1	8.9	8.8	0.3	0.2
Quercus mongolica ssp. *crispula*	Fagaceae	D	L, T, A	2.8	0.3	4.7	1.3	6.6	1.6	4.3
Angelica ursina	Umbelliferae	F	L, S	2.6	0.0	0.3	9.1	11.7		
Smilax china	Liliaceae	F	L, S, Fr	2.0	3.7	0.6	4.6	2.0		
Sorbus alnifolia	Rosaceae	D	L	2.0	1.5	5.8	1.1			
Spuriopimpinella calycina	Umbelliferae	F	L, S, Fl	1.6	2.6	2.1	2.5	0.9		
Carex blepharicarpa & *C. foliosissima*	Cyperaceae	G	L	1.5			3.9	4.8	1.6	2.0
Akebia trifoliata	Lardizabalaceae	D	L	1.4	0.6	0.9	5.1	2.8		
Viburnum furcatum	Caprifoliaceae	D	L, T	1.4	0.0	0.0			7.2	2.9
Viburnum wrightii	Caprifoliaceae	D	L, T	1.4	0.9	0.0		0.5	2.2	7.7
Pueraria lobata	Leguminosae	D	L	1.3	0.0	4.9	0.3			
Quercus dentata	Fagaceae	D	L, T	1.2	4.6				0.4	
Rhus ambigua	Anacardiaceae	D	L, T	1.2		2.1	2.6	0.4	0.2	1.6
Carpinus cordata	Betulaceae	D	L, T	1.1	2.1	0.0	0.0		2.5	2.1
Thujopsis dolabrata var. *hondae*	Cupressaceae	C	L	1.1			1.5	1.5	3.1	2.8
Berberis amurensis	Berberidaceae	D	L, Fr	1.1	1.0	2.3	1.6	0.8		
Angelica edulis	Umbelliferae	F	L, S	1.1	3.6	0.0	0.6		0.1	
Picrasma quassioides	Simaroubaceae	D	L, T	1.1	1.4	2.5	0.1	0.6		
Schizophragma hydrangeoides	Saxifragaceae	D	L, T	1.0	2.8	0.3	0.1	2.6		0.1
Stachyurus praecox	Stachyuraceae	D	L, T	0.9		0.3	0.0		5.4	0.4
Eupatorium chinense var. *oppositifolium*	Compositae	F	L, S, Fl	0.8	0.3	1.4	1.8	0.8	0.2	
Commelina communis	Caryophyllaceae	F	L, S, Fl	0.8		3.1				
Corylus sieboldiana	Betulaceae	D	L, T	0.8	0.6	1.4	0.3		1.4	0.4

Appendix 2.   (continued)

Species	Family name	Life form*	Part eaten**	Total	Spring Period A	Summer Period A	Autumn Period A	Autumn Period B	Winter Period A	Winter Period B
Lespedeza bicolor	Leguminosae	D	L	0.7	0.2	2.4	0.5			
Artemisia keiskeana	Compositae	F	L, S	0.7	0.3	1.9	1.0	0.5		
Fraxinus lanuginosa f. *serrata*	Oleaceae	D	L, T	0.6	0.5				1.6	2.2
Rubus crataegifolius	Rosaceae	D	L	0.6		0.3	2.3	1.5		
Actinidia arguta	Actinidiaceae	D	L	0.6	0.2	1.1	1.3	0.3		
Cirsium sp.	Compositae	F	L, S, Fl	0.6	0.2	0.8	1.4	0.9		
Kalopanax pictus	Araliaceae	D	L, Fr	0.6	2.1			0.3		0.0
Petasites japonicus	Compositae	F	L, S	0.5	0.1	1.7	0.4	0.1		
Cacalia auriculata var. *kamtschatica*	Compositae	F	L, S, Fl	0.5	0.4	0.6	0.5	1.7		
Zanthoxylum piperitum	Rutaceae	D	L	0.5		0.1		4.6		
Ampelopsis glandulosa var. *heterophylla*	Vitaceae	D	L	0.5	0.2	1.5	0.4			
Asperula odorata	Rubiaceae	F	L, S	0.5		0.1	2.8	0.2		
Evodiopanax innovans	Araliaceae	D	L	0.5	0.0	1.8				
Solidago virgaurea var. *asiatica*	Compositae	F	L, S, Fl	0.4	0.1	0.8	0.1	1.9		
Lonicera morrowii	Caprifoliaceae	D	L	0.4	0.0	1.1	0.6			
Adenophora remotiflora	Campanulaceae	F	L, S	0.4	1.5	0.1				
Epimedium koreanum	Berberidaceae	F	L, S, Fl	0.4	1.0	0.4	0.1	0.3		
Clematis stans	Ranunculaceae	D	L	0.4	1.4	0.0		0.2		
Trifolium repens	Leguminosae	F	L, S	0.3				3.0		
Ulmus laciniata	Ulmaceae	D	L	0.3	1.0	0.1				
Fagus crenata	Fagaceae	D	L, T	0.2	0.1				0.5	1.5
Morus australis	Moraceae	D	L, T	0.2	0.1	0.1		0.6	0.4	0.4
Boehmeria sylvestris	Urticaceae	F	L, S	0.1	0.0	0.0		1.3		
Persicaria thunbergii	Polygonaceae	F	L, S	0.1				1.4		
Cryptomeria japonica	Taxodiaceae	C	L	0.1			0.3		0.5	0.2
Aralia elata	Araliaceae	D	L	0.1	0.0			1.2		
Ligustrum tschonoskii	Oleaceae	D	L, T	0.1					0.5	
Alnus hirsuta var. *sibirica*	Betulaceae	D	T	0.0						0.3
Total				96.4	95.6	93.8	95.4	95.1	99.4	99.7
No. of observed bites				16,686	2,281	3,567	2,668	1,720	3,879	2,571

Life form*: D, deciduous broad-leaved tree (including six vine species); F, Forb; C, evergreen coniferous tree.
Part eaten**: L, leaf; T, twig with bud; S, stem; Fl, flower; Fr, fruit; A; acorn.

Synaptonemal complex analyses in the XY chromosomes of six taxa of *Clethrionomys* and *Eothenomys* from Japan

Masahiro A. Iwasa[1], Yoshitaka Obara[2], Eiji Kitahara[3] and Yoshiyuki Kimura[4]

[1,2]*Department of Biofunctional Science, Faculty of Agriculture and Life Science, Hirosaki University, Bunkyo-cho, Hirosaki 036-8561, Japan*
[3]*Forestry and Forest Products Research Institute, Ibaraki 305-8687, Japan*
[4]*Faculty of Education, Fukushima University, Fukushima 960-1296, Japan*

Abstract. The XY chromosomes of bone marrow metaphases and the XY-synapses of pachytene spermatocytes of six taxa of *Clethrionomys* and *Eothenomys* from Japan were examined using C-banding and surface-spreading techniques. Light, and electron, microscopy revealed that in the red-backed vole, the XY size ratios of the metaphase sex chromosomes and the SC-axes of pachytene XY-synapses show a similar pattern of variation. The X chromosomes of these vole taxa were classified, on the basis of their size and morphology, as one of two types, that is they were either acrocentric or sub-telocentric. Similarly, two types of Y chromosome, small and medium, were recorded. According to these criteria, *C. rufocanus*, *C. rutilus* and *Eothenomys andersoni* carry an acrocentric X chromosome and a small Y chromosome, whereas the two local forms of *E. smithii*, the so-called "*smithii*-type" and "*kageus*-type", carry a subtelocentric X chromosome and a medium Y chromosome. In contrast to these XY combinations, *E. imaizumii* showed a composite combination, with a subtelocentric X chromosome and a small Y chromosome. In view of earlier findings on the genetic background of *E. imaizumii* (Suzuki 1994; Suzuki et al. 1999), such a composite combination of the sex chromosomes suggests that *E. imaizumii* may have inherited an X chromosome from a female *E. smithii* and a Y chromosome from a male *E. andersoni* during the course of speciation through hybridisation.

Key words: red-backed voles, synaptonemal complex, X chromosome, Y chromosome.

In general, red-backed voles (*Clethrionomys* and *Eothenomys*) are karyologically conservative irrespective of their domestic and continental distribution and show very close karyotypic similarity (Rausch and Rausch 1975; Tsuchiya 1981; Obara and Yoshida 1985; Obara 1986; Modi 1987; Ando et al. 1988; Kashiwabara and Onoyama 1988; Modi and Gamperl 1989; Yoshida et al. 1989; Ando et al. 1991). Both diploid number and autosomal fundamental number were essentially 56 in all of the species examined. Interspecific chromosome variation of these voles is found only in the sex chromosomes (Tsuchiya 1981; Yoshida et al. 1989;

Ando et al. 1991; Kitahara and Harada 1996; Iwasa 1998), except for one case of a 1–9 translocation in a lineage containing *C. glareolus*, *C. rutilus*, *C. gapperi* and *C. californicus* (Gamperl 1982; Modi and Gamperl 1989; Obara et al. 1995).   Such interspecific variation of the sex chromosomes is unclear among these voles and the synaptonemal complex (SC) analysis at the electron microscopic level, as well as a detailed chromosome banding analysis at the light microscopic level, may be necessary to clearly define interspecific variations of the sex chromosomes of this group.

Suzuki (1994) and Suzuki et al. (1999) found, based on the RFLP (restriction fragment length polymorphism) analysis of the rDNA spacer region, that *E. imaizumii* carries two types of rDNA in about equal amounts in its genome, one the *E. andersoni* type and the other the *E. smithii* type.   This mixture indicates that perhaps this species is of hybrid origin involving both *E. andersoni* and *E. smithii*.   In addition, Kitahara and his colleague examined in detail the taxonomic allocation of three local populations of Anderson's red-backed voles *andersoni*, *niigatae* and *imaizumii* on the basis of morphological and developmental analyses and crossbreeding experiments, and concluded that these three taxa belong to one species *E. andersoni*, and that "*imaizumii*" is phylogenetically closer to *E. smithii* than it is to *C. rufocanus* (Kitahara 1995a, 1995b; Kitahara and Kimura 1995).   In view of these findings, it would appear that "*imaizumii*" is closely related, genetically as well as morphologically, to both *E. andersoni* and *E. smithii*.

In order to test this view from a cytogenetic standpoint, we describe an analysis of X and Y chromosomes in mitotic metaphase and the SC configurations of XY-synapses in meiotic prophase from six taxa of *Clethrionomys* and *Eothenomys* from Japan, paying special attention to the XY combination of *E. imaizumii*.

Materials and methods

Animals

In total, 36 male specimens of two *Clethrionomys* species, *C. rufocanus* (Crf) and *C. rutilus* (Crt), and three *Eothenomys* species, *E. andersoni* (Ean), *E. smithii* and *E. imaizumii* (Eim) were used in this study.   Smith's red-backed vole, *E. smithii*, consists of two geographical forms, the "*smithii*-type" (Esm) distributed west of the Chubu district, and the "*kageus*-type" and Ekg), distributed east of the Chubu districts (see Table 1 for a summary of the collecting details).

Species were identified on the basis of the salient angle pattern of the third upper molars, following Kaneko (1994).   Although Kaneko (1994) and Kitahara and Kimura (1995) regarded Eim and Ean to be conspecific, the "*andersoni*" population from the Kii Peninsula has been dealt with here as a distinct species, *E. imaizumii*, following Jameson (1961), Imaizumi (1988), and the Japanese Environmental Agency (1993).

Somatic chromosome analysis

Somatic chromosomes were obtained from the bone marrow cells of femurs, using the short-term culture method (Obara 1982).   After 30 min colchicine treatment (0.03 μg/ml at the final concentration) in TC199 medium supplemented with 15% calf serum, the cells were incubated in 0.075 M KCl solution at 37°C for 18 min.   The cells were spread on glass slides and air-dried under moist conditions after being fixed with Carnoy's fixative

Table 1. Species of Japanese red-backed voles examined in this study.

Species	Collecting locality	Specimen's No.
Clethrionomys rufocanus	Oiwake, Hokkaido	94Crf-1; 95Crf-3
	Onnetoh, Ashoro, Hokkaido	94Crf-1; 95Crf-1, 2
Clethrionomys rutilus	Shunkunitai, Nemuro, Hokkaido	94Crt-1, 2, 6, 9, 14, 15
	Bunsen, Rikubetsu, Hokkaido	94Crt-21
	Onnetoh, Ashoro, Hokkaido	94Crt-23, 24
	Abiragawa, Tomakomai, Hokkaido	95Crt-2, 5
	Chitose, Hokkaido	95Crt-4
Eothenomys andersoni	Zatoh-ishi, Hirosaki, Aomori Pref.	94Ean-4, 6, 8; 95Ean-1
	Tennohzawa, Hirosaki, Aomori Pref.	95Ean-2, 4
	Mt. Iwaki, Iwaki, Aomori Pref.	95Ean-3
Eothenomys smithii	Kamikumeda, Matsuoka, Fukui Pref.	94Esm-1, 3, 6; 95Esm-5
	Matogawa, Matsuoka, Fukui Pref.	94Esm-2
	Eiheiji, Eiheiji, Fukui Pref.	95Esm-1
	Kotakakura, Ohtama, Fukushima Pref.	94Ekg-4, 5; 95Ekg-1
Eothenomys imaizumii	Owase, Mie Pref.	95Eim-1, 2, 3

(methanol : acetic acid = 3 : 1). Air-dried chromosomes were differentially stained for C-banding, following Sumner's (1972) BSG method. From 15–40 cells from each species of voles were examined, and the relative lengths of their X and Y chromosomes and the XY ratio were analysed.

Synaptonemal complex analysis

Synaptonemal complex (SC) preparations of the spermatocytes were made from testicular materials taken from adult males following the slightly modified methods of Moses (1977) and Greenbaum et al. (1986). The SC and unsynapsed axes of XY-synapses were stained following Howell and Black's (1980) one-step method. The silver-stained preparations were observed with transmission electron microscopes (Nippon Denshi JEM-1210 80 kV and Hitachi H-600 75 kV), and photographed in order to measure the actual length of SC plus the unsynapsed axis (SC-axis). Measurement of the length of the XY-synapses were made using the IP Lab Spectrum program (Signal Analytic Corporation) after scanning the SC-axis with an image scanner (EPSON GT-8500). From 12 to 36 SC-axes were studied, and their absolute lengths, mean lengths and standard errors for the full pachytene stages (including early-, mid- and late-pachytene substages; subdivided according to Greenbaum et al. (1986)), were analysed statistically.

Results

The autosomes of the red-backed voles examined, with the exception of Crt which is characterised by 1–9 translocations (Modi and Gamperl 1989; Obara et al. 1995), showed no interspecific variation in their G-banding pattern, confirming the earlier reports of Obara and Yoshida (1985) Ando et al. (1988), Yoshida et al. (1989), Ando et al. (1991), and Kitahara and Harada (1996).

The X chromosomes of these voles were classified as either acrocentric (Crf, Crt and Ean) or subtelocentric (Esm, Ekg and Eim), while the Y chromosomes were defined as
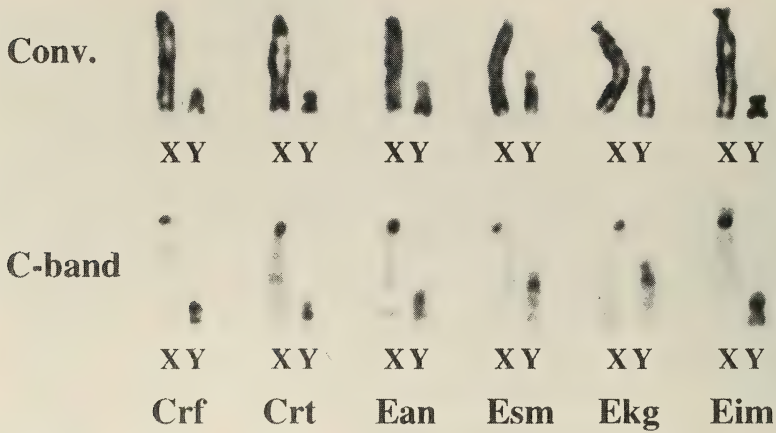
Fig. 1.   Conventionally-stained (upper) and C-banded (lower) XY chromosomes from the bone marrow metaphases of the red-backed voles examined.   Crf=*C. rufocanus*; Crt=*C. rutilus*; Ean=*E. andersoni*; Esm=*E. smithii* (*smithii*-type); Ekg=*E. smithii* (*kageus*-type); Eim=*E. imaizumii*.

"small" (Crf, Crt, Ean and Eim) or "medium" (Esm and Ekg) (see Fig. 1).   The subtelocentric type X chromosomes were relatively longer than the acrocentric types, and the short arm seemed to be euchromatic on the basis of its negative C-band staining properties (Fig. 1). A deeply stained centromeric C-band was observed in both types.   The "small" Y chromosome, varied in morphology (acro-, submeta- and metacentric) among the various vole species, but had a centromeric C-band in all taxa examined.   The remaining interstitial and terminal areas of the "small" Y chromosome were C-stained less darkly than the centromeric C-band.   In Crt, the Y chromosome was deeply C-stained along its entire length.   The "medium" Y chromosome was subtelocentric, and deeply C-stained from the centromeric to the proximal area (Esm and Ekg) of its long arm.   Crf, Crt and Ean carried an acrocentric X chromosome and a small Y chromosome, whereas Esm and Ekg both carried a subtelocentric X chromosome and a medium Y chromosome.   Unlike these XY combinations, the sex chromosomes of Eim were composed of a subtelocentric X chromosome and a small Y chromosome.   The X chromosome of Eim seemed to be slightly longer in its short arm when compared with that of Esm and Ekg, and was the longest X chromosome among the six vole taxa examined.   The Y chromosome of Eim was closer in length to that of Ean than that of Crf and Crt.   The mean XY ratios in the bone marrow metaphases of Crf, Crt, Ean, Esm, Ekg and Eim were 0.25, 0.22, 0.27, 0.43, 0.46 and 0.26, respectively.   It is unclear whether such variations in the length and ratio of the XY chromosomes are significant since chromosome condensation is variable.

Actual measurements of surface-spread XY-synapses in full pachytene may provide more reliable information about chromosomal length.   In general, autosomal pachytene SCs are formed along the entire length of the corresponding autosomal homologues.   Thus, the length of a given autosomal pachytene SC reflects that of the corresponding autosome in pachytene.   However, in a surface-spread XY-synapsis, SC is usually only partially formed, between a small region of the X chromosome and a small region of the Y chromosome, as
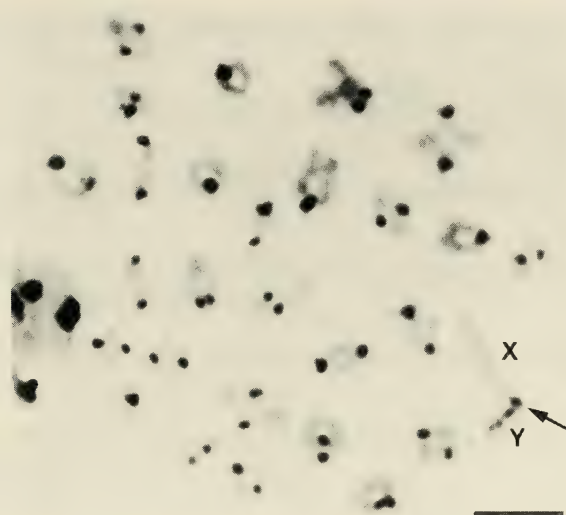
Fig. 2. C-banded metaphase I plate in a spermatocyte of *E. andersoni*. Arrow indicates association between centromeric end of the X chromosome and one end of the Y chromosome. Bar=5 μm.

suggested from a typical example of a C-banded XY bivalent in the MI stage of Ean (Fig. 2). The remaining unsynapsed areas form rather knobby axes (X- and Y-axes), as in most mammalian species reported so far (Moses 1977; Solari and Rahn 1985; Schmid et al. 1987; Sudman and Greenbaum 1990; Villagomez 1993; Ashley and Fredga 1994; Iwasa and Obara 1995). A full set of SC configurations of a surface-spread pachytene spermatocyte from Ean is shown in Fig. 3a, and typical examples of XY-synapsis in pachytene from the six taxa examined are shown in Fig. 3b–3g. In Esm and Ekg, the Y chromosome synapses with the X chromosome along less than one fifth of its entire length, and forms the SC in the synapsing area, whereas in the remaining four species the SC forms along almost half of the entire length. The long X-axis often turns round, crosses over itself (Fig. 3c, d, f and g), and sometimes forms an end-to-end association with a terminal end of the Y-axis (Fig. 3e). The statistical results of axes measurements are shown in Fig. 4. As in the metaphase chromosomes, the length of the SC-axis of the X chromosome (SC-axis (X)) in the XY-synapsis was distributed in two distinct groups, small and large: $16.07\pm0.56\,\mu$m$\sim17.11\pm0.78$ μm (Crf, Crt and Ean) and $18.50\pm0.55\,\mu$m$\sim20.10\pm0.64\,\mu$m (Esm, Ekg and Eim). The SC-axis length of the Y chromosome (SC-axis (Y)) also occurred in small and medium groups: $3.62\pm0.15\,\mu$m$\sim5.13\pm0.17\,\mu$m (Crf, Crt, Ean and Eim) and $7.93\pm0.46\sim9.20\pm0.86$ μm (Esm and Ekg). Thus, Crf, Crt and Ean carry small XY chromosomes, whereas Esm and Ekg carry medium or large XY chromosomes. In contrast, the XY-synapsis of Eim was composed of a large X chromosomes and a small Y chromosome. The mean XY ratios of the SC-axis in the pachytene spermatocytes of Crf, Crt, Ean, Esm, Ekg and Eim were 0.31, 0.21, 0.26, 0.43, 0.48 and 0.26, respectively, roughly matching the mean XY ratios in the bone marrow metaphases.
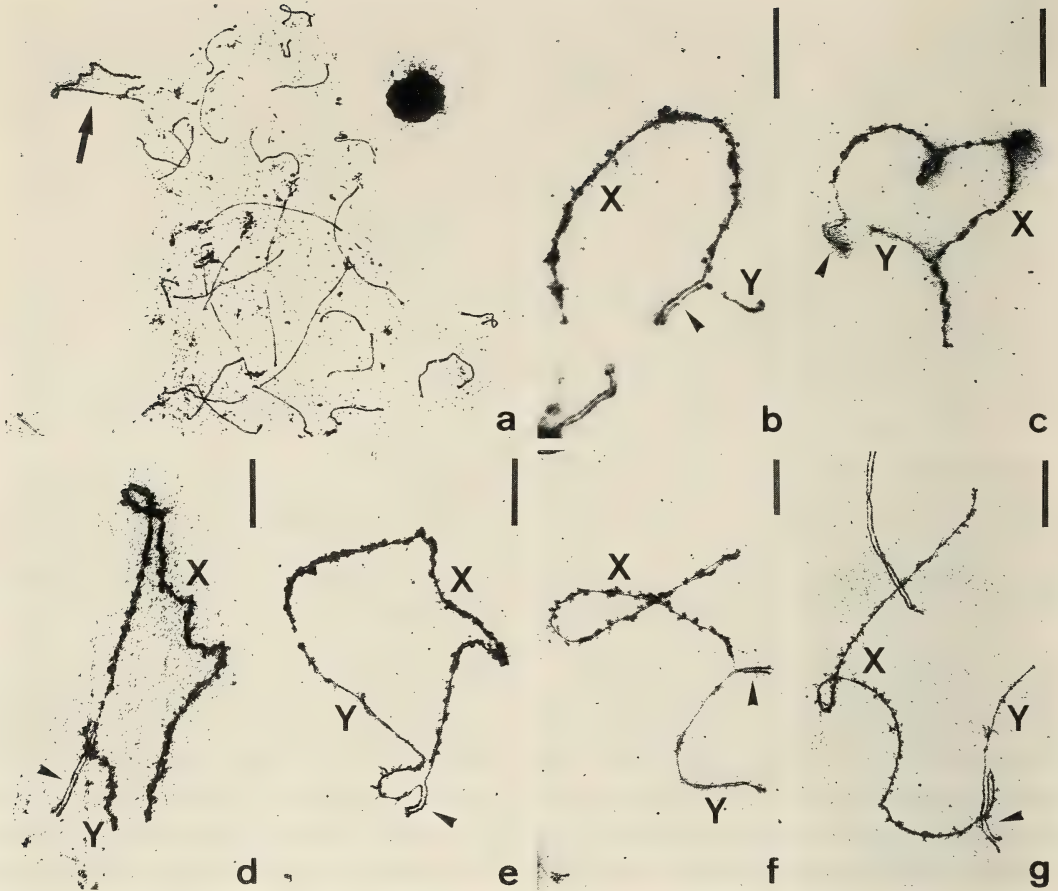
Fig. 3. Electron micrographs of a surface-spread pachytene spermatocyte of *E. andersoni* (a) and magnified XY configurations (b–g) in the pachytene spermatocytes of the red-backed voles examined. The arrow indicates the XY-synapsis and arrowheads indicate SC regions. X and Y show X- and Y-axes. b=*C. rufocanus*; c=*C. rutilus*; d=*E. andersoni*; e=*E. smithii* (*smithii*-type); f=*E. smithii* (*kageus*-type); g=*E. imaizumii*. Bar=2 µm.

Discussion

The mitotic chromosomes of the red-backed voles examined were quite conservative in their autosomes, with the exception of 1–9 translocations in Crt, whereas the sex chromosomes generally showed interspecific variations in both the size and the morphology of the X and Y chromosomes, as in other species in this group (Rausch and Rausch 1975; Nadler et al. 1976; Modi 1987; Kashiwabara and Onoyama 1988; Modi and Gamperl 1989; Yoshida et al. 1989). In such an autosomally conservative group, the sex chromosome morphology, therefore, is the only marker that distinguishes between the species chromosomally. These findings indicate that the two vole genera, *Clethrionomys* and *Eothenomys*, are phylogenetically close to each other. Furthermore, the G-banding patterns of the long arms of the X chromosomes of these voles showed good accordance with each other (Obara 1986; Tsuchiya et al. 1986; Ando et al. 1988; Modi and Gamperl 1989; Yoshida et al. 1989; Obara
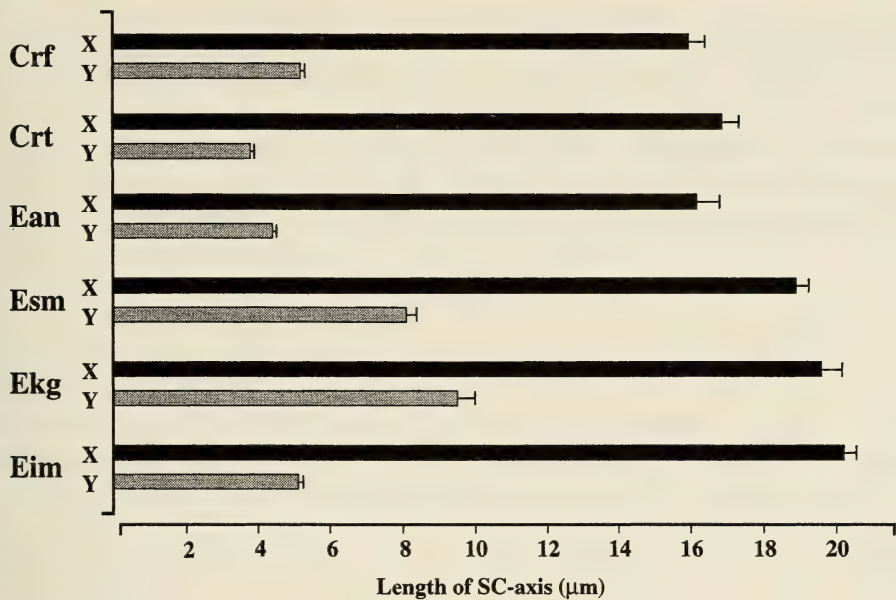
Fig. 4. Actual lengths of SC-axes (X and Y) in the surface-spread pachytene spermatocytes of the red-backed voles examined. Thick and thin lines indicate mean length and standard error, respectively.

et al. 1995; Kitahara and Harada 1996), therefore, it is considered that the difference of the X chromosomal morphology among these voles might have arisen as a result of the presence or absence of the short arm. Thus, the morphological differences between the subtelo- and acrocentric X chromosomes seem to have been formed through minor chromosomal rearrangements, such as the addition or deletion of the short arm segment. In this context, Kitahara and Kimura (1995) crossbred Anderson's red-backed voles (Eim) from the Kii Peninsula with Anderson's red-backed voles (Ean and *E. niigatae*; formerly *Clethrionomys andersoni* and *C. niigatae*) from Fukushima and Nagano Prefecture, obtaining fertile hybrids in either combination, and concluded, supporting Aimi's (1980) opinion that Eim, Ean and *E. niigatae* are all conspecific. Although their crossbreeding experiments were reliable and significant, our chromosome and SC analyses revealed that Eim is apparently different from Ean in the size and morphology of the X chromosome, suggesting no genetic interchange between Eim and Ean. In fact, Eim which inhabits the restricted area of the Kii Peninsula has been geographically isolated from Ean, which ranges from the Chubu to the Tohoku districts. It remains unknown whether Eim and Ean are able to produce fertile hybrids in the wild.

In the Chinese hamster *Cricetulus griseus*, there is a 1 : 1 relationship between the relative lengths of autosomal SCs and mitotic autosomes. The unpaired X- and Y-axes of pachytene spermatocytes shorten and lengthen, however, not necessarily matching in relative lengths the sex chromosomes of bone marrow metaphases, though the SC portion of XY-synapsis is constant in length through most of the pachytene (Moses et al. 1977). This phenomenon is believed to be caused by the presence of a high amount of C-heterochromatin contained in the X and Y chromosomes. In all the vole species examined here, the X chromosome had a small amount of C-heterochromatin, which is detected as a centromeric

C-band, and the Y chromosome had a deeply stained centromeric-to-proximal C-band and a slightly lighter whole arm C-heterochromatin. If this view is correct, the variation in length of the X- and Y-axes of pachytene spermatocytes should be small in vole species, and therefore the XY-ratio of the SC-axis in pachytene should also be close to that of the sex chromosomes during metaphase. This relationship seems to be roughly compatible in all vole taxa examined. Thus, there may be a close relation, at least in the vole species examined, between the XY-ratios in pachytene spermatocytes and those in bone marrow metaphases. Moreover, the XY-synaptic regions of Crf, Crt, Ean and Eim were longer than those of Ekg and Esm (Figs. 3b–g). The differences in the synaptic conditions among these vole taxa may be have arisen naturally at the pairing region of the Y chromosome. Thus, it is considered that the differences in synaptic lengths between X and Y axes among the various combinations of the XY chromosomes (such as acrocentric X – small Y, sub-telocentric X – small Y, and subtelocentric X – medium Y), lead to that synaptic property dependent on the nature of the segment in the synaptic region of the Y chromosomes.

Our chromosome and SC analyses clearly demonstrate that three vole species Crf, Crt and Ean have an acrocentric X – small Y combination (a small XY chromosome type) and two geographic forms Esm and Ekg have a subtelocentric X – medium Y combination (a large-medium type of XY) (Figs. 1 and 4). Of significance is the finding that the sex chromosomes of Eim were composed of a subtelocentric type X and a small type Y (a composite type of XY) (Figs. 1 and 4). In the light of these findings, Eim shares the X chromosome with the Esm – Ekg group, yet shares the Y chromosome with the Crf – Crt – Ean group. Therefore, it is tempting to consider that the X chromosome of Eim may have originated from the Esm – Ekg group and the Y chromosome from the Crf – Crt – Ean group. No significant difference in length of the SC-axis (X) was found among Esm, Ekg and Eim, whereas the SC-axis (Y) of Eim was statistically different in length from that of Ean, but not from that of Crf ($P<0.05$). So, the data from the XY-synapsis may suggest inheritance of the X chromosome from either Esm or Ekg, and the Y chromosome from Crf. However, the latter species has been isolated in Hokkaido, thus having been widely separated from the Kii population, and similarly Ekg is distributed east of the Chubu districts. Taking such geographic distribution into consideration, it is most likely that the X and Y chromosomes of Eim has been inherited, through speciation by hybrid formation, from Esm and Ean, respectively, even though inequality of the SC-axis (Y) between Eim and Ean still leaves much to be explained.

Recently, Suzuki (1994) and Suzuki et al. (1999) studied the rDNA of several vole species including Eim by RFLP analysis, and found two types of rDNA, or Ean type and Esm – Ekg type in its genome in equal proportions. Based on their molecular findings they proposed that the ancestral form of Eim (probable Ean-type) had been distributed on the Kii Peninsula and that genetic interchange has occurred recently between Esm – Ekg and the ancestral form. Our evolutionary scenario of Japanese red-backed voles, in particular for Eim, suggests that Eim might be derived from hybridization between the ancestors of Ean and Esm on the basis of molecular phylogenetic findings (Suzuki 1994; Suzuki et al. 1999), and present data on the sex chromosome constitution of Eim is well compatible with these molecular viewpoints. Furthermore, the Y chromosomes of Crf, Crt, Ean and Eim are cytogenetically quite similar, on the basis of morphological and differential staining criteria (Tsuchiya 1981; Obara 1986; Tsuchiya et al. 1986; Ando et al. 1988; Yoshida et al. 1989; Obara et al. 1995;

Kitahara and Harada 1996) because the Y chromosomes of Ekg and Esm were more differentiated from the cytogenetic aspect in the voles (Yoshida et al. 1989; Hielscher et al. 1992; Iwasa and Tsuchiya unpublished data) including related species. From cytogenetic and other phylogenetic aspects, small Y chromosomes might occur through minor chromosomal rearrangements such as inversion, however, medium Y chromosomes carrying partially heterochromatic segments might be derived from more confused rearrangement than small ones. Further research focussing on the sex chromosome-linked genes such as *Sry* may provide a way of disclosing this issue.

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A record of the food retention time of the Asiatic elephant, *Elephas maximus*

Udayani R. Weerasinghe[1], Palitha Jayasekara[2] and Seiki Takatsuki[3]

[1]*Laboratory of Wildlife Biology, School of Agriculture and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, 113-0032 Tokyo, Japan*
[2,3]*The University Museum, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, 113-0033 Tokyo, Japan*

We carried out a brief feeding experiment in order to measure the food retention time of the Asiatic elephant, *Elephas maximus*. For the experiment, a tame individual of the endemic Sri Lankan subspecies, *E. m. maximus*, was used. Compared with the African elephant, *Loxodonta africana*, the biology of the Asiatic elephant is poorly known, although there have been classic studies on its physiology (Benedict 1936) and ecology (Sukumar 1989). Because of economic development, the Sri Lankan elephant, whose present population is estimated to be 3,000 or 4,000 (Santiapillai and Jackson 1990; Jayewardene 1994), is confined in small areas. Because of their large body size, they sometimes heavily affect the vegetation of their habitat. For the better management of the elephants and their habitat, therefore, an understanding of the food-related biology of the Asiatic elephant is important.

Food retention time has been studied in both African (Bax and Sheldrick 1963; Rees 1982) and Asiatic elephants (Benedict 1936). However, previous studies have only shown the elapsed time after intake to the first and last excretion of markers in the dung. During the present study, based on the exact excretion pattern of two types of markers, we were able to measure the elapsed time from the first intake to the first excretion, the time to peak of excretion, the time to last excretion, and the mean retention time.

Materials and methods

A 48-year-old tame male elephant was used for the feeding experiment. Based on the correlation between shoulder height and body weight of the Asiatic elephant (McKay 1973), this individual, which stood 265 cm high at the shoulder, was estimated to weigh 3.8 tons. The elephant was kept outside, and chained by his hind foot to a tree. Nearby ground-covering vegetation was cleared away and known amounts of forage were given as food. He was walked once a day to a pond, where he was able to drink water and bathe.

Benedict (1936) and Vancuylenberg (1977) both reported that an Asiatic elephant eats about 150 kg of foods per day. On this basis, during the course of our experiment, from 19th to 26th May 1998, the tame elephant was fed 300 kg of fresh food at 15:00 everyday, which should have been more than sufficient for its needs. This consisted of 150 kg of leaves and twigs of Jak, *Artocarpus heterophyllus*; 100 kg of palm woods, *Caryota urens*; and 50 kg of grasses, mainly *Panicum maximum*. On the second day of the experiment, a piece of bread, inside which 1,000 plastic beads were hidden, was added to the daily ration. The

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[3]*To whom correspondence should be addressed.   E-mail: taka@um.u-tokyo.ac.jp*

beads were about 2 mm in diameter and 3 mm long.   On the third day, five melons were given as supplemental food as these contain many seeds.   The melon seeds were flat and spindle-shaped, measuring 4 mm in width, 6 mm long and 1 mm thick.

All of the elephant's dung was collected at 08:00, 12:00 and 18:00.   Each dropping was weighed to the nearest gram using a kitchen balance.   In addition, dung was collected as frequently as possible, whenever the elephant was observed defecating.   We sampled the collected dung, breaking the droppings in order to count the number of seeds and beads they contained.   As most of the beads were found to have been completely or partly destroyed, presumably by mastication, we counted only beads which maintained more than half of their original size, and ignored smaller portions.   The weight contribution of the dung samples to the total dung was 43.5%.   After the peaks of the appearance of the two kinds of markers, we extended the interval of fecal collection.   As the intervals between dung collection were not constant, but longest during the night, seed and bead numbers recovered from the sample dung were converted to a total number according to dry weight, and then divided by the time interval (hr) of collection.   We assumed that the density of markers in dung during each interval was constant.

The mean retention time (*MRT*) was calculated by the formulae:

$$MRT = \Sigma(M \times T)/\Sigma M$$

Where T is the length of time between dosing and excretion in the feces, and M is the total amount of marker in that collection (Coombe and Kay 1965).

Results

The process of excretion of the dietary markers was expressed as a percentage of the number of markers recovered at collections to the total number voided.

Melon seeds first appeared 14 hrs after ingestion, and quickly increased to reach a sharp peak (40.1% of the total number of excreted seeds) at 18 hrs after ingestion (Fig. 1).   The seed numbers then decreased to 11.0%, and thereafter it gradually decreased until 48 hrs after dosing.   The last seed was found 72 hrs after ingestion, though the single seed was found from over 5 kg of dung.

The pattern of the passage of beads was similar to that of passing melon seeds.   The beads first appeared in dung 17 hrs after ingestion, and rapidly increased to reach a peak at 25 hrs after ingestion.   The peak, however, was not as sharp as found for melon seeds, and the decrease following the peak was also more gradual (Fig. 1).   The continuous voiding of the beads continued up to 63 hrs after ingestion.   Thereafter, no beads appeared for a while, but then one bead appeared at 73 hrs after dosing when we ended the collection.   The mean retention time was calculated as 20.2 hrs for melon seeds and 29.7 hrs for beads.

A total of 311 beads were recovered.   From the total weight of dung voided, it was estimated that 714.9 beads (71.5%) appeared during the experiment, indicating that 28.5% were destroyed during the digestion process, probably mainly during mastication.
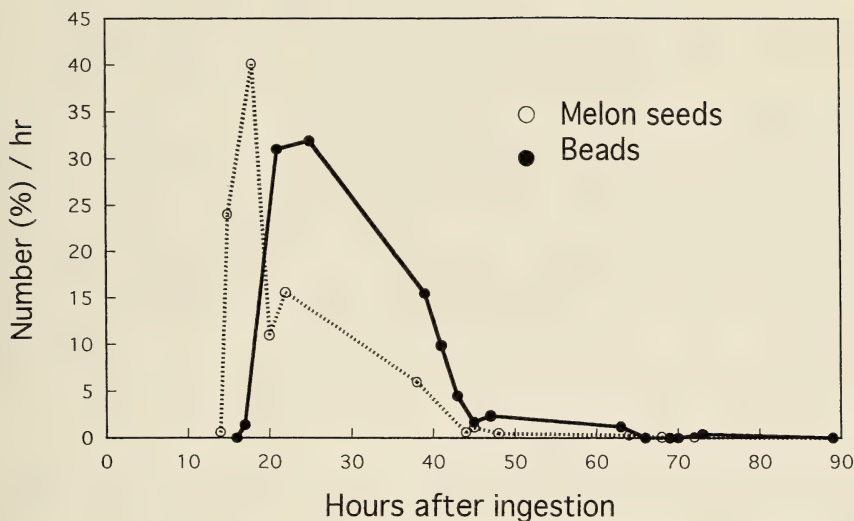
Fig. 1. Numbers of beads and melon seeds recovered from dung of an Asiatic elephant after ingestion. The numbers are percentages of the total numbers recovered.

Discussion

Description of the experiment

As soon as food was provided at 15:00 each day, the elephant began to eat, and clearly preferred the marker foods (the bread containing the beads, and the melons). During the first hour he ate great amounts of food, and thereafter fed at a more leisurely pace throughout the night (Somaratna personal communication). We did not see him eating in the mornings when we resumed dung collection at 08:00, which suggests that he was satisfied after feeding during the night. The average fresh weight (±SD) of his daily intake was 123.8 (±27.5) kg (Weerasinghe et al. unpubl.), which was similar to the amounts described in previous studies (McKay 1973; Vancuylenberg 1977).

Markers

It is known that retention times are different among different foods (Rees 1982). In this study, the bead markers appeared, and peaked, later than the melon seeds. The markers differed in two ways. They differed firstly in their quality. Since the beads were made from plastic, they were non-digestible despite having been heavily masticated and broken into smaller pieces, and this may have contributed to them being passed more slowly than the melon seeds. However, since we did not count the number of melon seeds in advance of the feeding experiment, the differences in the digestibility between the two markers is not known. Secondly, the difference in size and shape of the markers may have also affected their passage. The flatter shape of the melon seeds, together with the jelly-like attachment around them, may have facilitated their rapid passage, despite the fact that they were slightly larger than the beads. Relative weight may also affect passage rates. It seems possible that, because of their small size, if they were light, they would be passed to the

lower tracts with liquids faster than fibrous plant materials.   However, we did not measure the relative weights of the two markers.

Pattern of passage

Previous studies of through-put times for elephants have concentrated simply on showing the first and the last appearance of dietary markers (Benedict 1936; Bax and Sheldrick 1963; Rees 1982).   Benedict (1936) fed rubber pieces to an Asiatic elephant and determined that the first and the last appearances were about 24 and 51 hrs after ingestion, respectively. Bax and Sheldrick (1963) briefly mentioned that an African elephant excreted orange seeds between 11 and 14 hrs after feeding and continued to produce them up to 19 hrs later.   Rees (1982) fed beetroot to an African elephant and found that passage took between 21 and 46 hrs.   This study then, is the first that has examined the patterns of excretion of dietary markers.   The peaks of passage occurred between 17 and 25 hrs after ingestion.

The retention time of the Asiatic elephant seems to be shorter than ruminants, when taking in to account their body size.   The mean retention times of ungulates, which are much smaller than elephants, were 15–30 hrs for sheep, *Ovis aries* (Coombe and Kay 1965), 20–40 hrs for the white-tailed deer, *Odocoileus virginianus* (Mautz 1971), 35–45 hrs for the goat, *Capra hircus* (Castle 1956), and 70–90 hrs for cattle, *Bos taurus* (Balch 1950).   Retention time, or the rate of passage of food through the digestive tract, can be expected to depend on the length of the digestive tract, and as ruminants have very long intestines, it is not surprising, therefore, that their retention times are very long for their body sizes.   Unfortunately, no information is available on the length of the digestive tract of elephants.   The short retention time of the Asiatic elephant may result from it having a simple stomach, and short intestines (Clements and Maloiy 1982).   This simple digestive system seems to enable the ingesta to pass through the tracts rapidly, though the African elephant at least has a well-developed colon and caecum (Clements and Maloiy 1982).   In contrast, ruminants have complex stomachs where the ingesta are retained and fermented, and hence food is passed very slowly through the alimentary canal.

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Seasonal change in reproductive states of the Formosan squirrel on Izu-Oshima Island, Japan

Noriko Tamura[1]

Tama Forest Science Garden, Forestry and Forest Products Research Institute, Todori 1833, Hachioji, Tokyo 193-0843, Japan.

The Formosan squirrel, *Callosciurus erythraeus thaiwanensis*, was introduced to Japan and now occurs in several areas of the country (Yamaguchi 1988; Kawamichi 1997).   The breeding season of this species has been investigated in Taiwan, ROC, where it is indigenous. There, dissection of 100 females revealed that although they reproduce throughout the year there are two peaks, from January to March, and again from May to August (T'sui et al. 1982).   In Kamakura, Japan, where the same species has been introduced, mating behavior has been observed throughout the year, but with two peaks, the first during March and April and the second from July to September (Tamura et al. 1988).   In captivity, the gestation period of the Formosan squirrel is 47–49 days, and the young squirrels leave the nest 40–50 days after birth (Tamura and Terauchi 1994).   On the basis of these captive studies, weaned juveniles can be expected to appear in the wild during July and August, and during the period from November to January.   This expectation is borne out, with juveniles weighing less than 200 g (just after weaning) being captured in live-traps most frequently during these months (Tamura 1989).

Formosan squirrels have also been introduced to Izu-Oshima (34°45′N, 139°22′E), an island of approximately 9,100 ha south of Tokyo.   Since their introduction there ca. 60 years ago they have increased rapidly and spread all over the island with the exception of the unvegetated volcanic areas.   Because the squirrels feed on *Camellia* seeds and other agricultural products on the island, they are considered a pest and several hundreds individuals have been killed each year since 1970.   The reproductive strategy of Formosan squirrels on Izu-Oshima has not previously been studied, so here I will present data on the breeding cycle of this species on this island, which may prove helpful in controlling the population.

Methods

Squirrels shot as pests during the period from November 1996 to February 1997, from May to October 1997, from February to April 1998, and from October to November 1998 were examined for this study.   The squirrels had been shot at sites scattered across the island, with the exception of the area around the crater of Mt. Mihara in the center of the island, and the volcanic eastern part of the island.   All specimens were sexed and weighed immediately after shooting, then frozen and transported to the laboratory for analysis. After subsequent thawing of the specimens, the snout-vent length of all females was

[1]*E-mail: haya@ffpri.affrc.go.jp*

measured, and the condition of the teats and vaginal opening were recorded. The teats were defined as either projecting or not projecting, and used as an indication of whether females were lactating or not. The stage of estrous was assessed on the basis of the color of the vaginal opening. During the period 5–7 days before copulation the vaginal opening became pink and swollen whereas most of the time it was small, inconspicuous, and gray in color.

More detailed analysis was made of the ovaries and uterine horns after they had been dissected out. Ovaries were sectioned longitudinally and the stage of developmental of the follicles was observed under a binocular microscope ($\times 80$). Four stages of sexual maturity were recorded using T'sui et al.'s (1982) criteria. These were: 1) mature (ovary with Graafian follicles, and with projecting teats); 2) young mature (ovary with Graafian follicles, but non-projecting teats); 3) subadult (ovary with several secondary solid follicles and a few secondary vesicular follicles), and 4) immature (ovary with only primary follicles). The number of fetuses found in the uterine horns was also recorded.

Results and discussion

Of the total of 190 females, 132 with projecting teats, indicating that they had experienced lactation, were defined as mature. The mean body weight of mature females was 376.5 g$\pm$29.6 (*SD*); range 320–440 g, $n=132$). The mean snout-vent length of the same females was 21.06 cm$\pm$0.88 (*SD*); range: 19.5–23.3 cm, $n=132$). Forty-three of the mature females were carrying fetuses in their uterine horns.

The remaining 58 females examined had non-projecting teats, however, one of them had fetuses in the uterus, and 10 of them had swollen vaginae, indicating that they were in heat. As these 11 individuals all had Graafian follicles in their ovaries, they were defined as young mature females. The mean body weight ($\pm SD$) of these young mature females was 318.3 g$\pm$36.2 (range: 280–390 g, $n=11$), and their snout-vent length averaged 20.7 cm$\pm$0.7 (range: 19.5–21.5 cm, $n=11$).

Seven females with non-projecting teats and vesicular follicles in their ovaries were defined as subadults. They averaged 325.0 g$\pm$22.9 (*SD*) (range: 300–360 g, $n=7$) and measured 20.4 cm$\pm$0.8 (*SD*) (range: 19.0–22.0 cm, $n=7$) from snout to vent.

The remaining 40 females with non-projecting teats, had no vesicular follicles in their ovaries, so they were defined as immature. The mean body weight ($\pm SD$) of these immature

Table 1. The reproductive state of female squirrels each month. Numerals in parentheses indicate the number of females in estrous.

Reproductive States	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Mature	4	22	9	7	4	22	7	19	8	11	10	9
	(0)	(5)	(0)	(0)	(0)	(6)	(3)	(1)	(1)	(1)	(0)	(3)
Young mature	0	0	1	1	1	4	2	0	0	1	1	0
	(0)	(0)	(1)	(1)	(1)	(3)	(2)	(0)	(0)	(0)	(1)	(0)
Subadult	0	0	0	0	0	0	0	1	0	1	3	2
Immature	4	2	4	4	3	5	3	6	1	2	4	2
Total	8	24	14	12	8	31	12	26	9	15	18	13

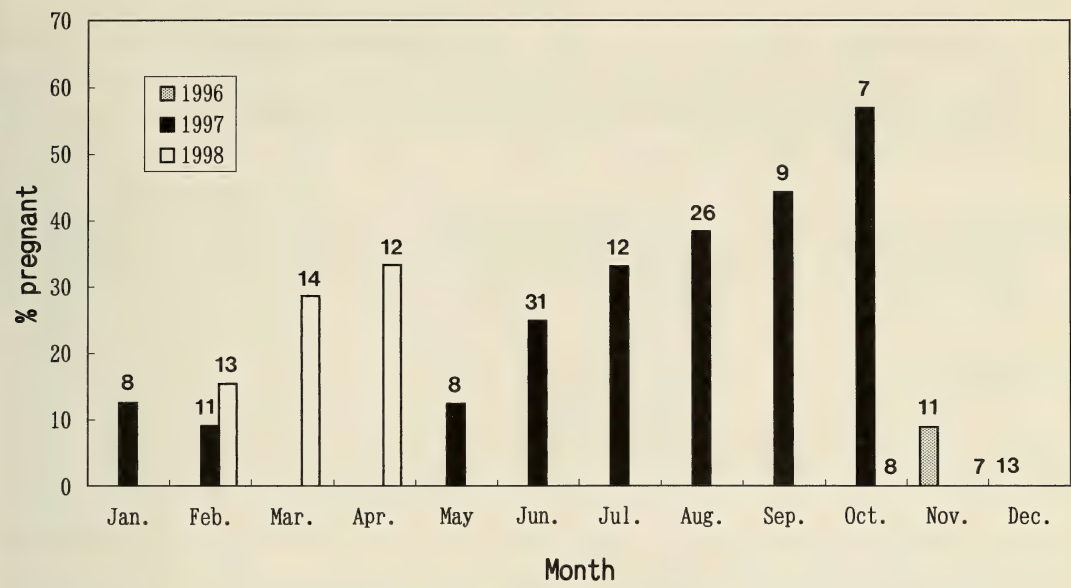**Fig. 1.** Seasonal changes in the percentage of pregnant females. Numerals above bars indicate sample sizes.

females was 298.8 g±42.2 g (range: 195–350 g, $n=40$), and they averaged 19.9 cm±1.3 (*SD*) (range: 17.0–22.0 cm, $n=40$) from snout to vent.

There were no apparent seasonal changes in the proportion of squirrels in each of the four reproductive states (Table 1). The proportion of immature females in relation to the total number of females caught each month, showed no seasonal trend, and ranged from 8% to 50% with a mean of 23.6%. This proportion was similar to that found in Taiwan (20.5%; T'sui et al. 1982). Although the sample size was small, females in estrous were captured more frequently during February, June and July than in other months (Table 1).

A total of 44 females was found with pregnancies recorded in every month except December. The pregnancy rate was highest during March to April and again from July to October (Fig. 1). These seasonal trends in pregnancy rates were coincident with the number of estrous females. The seasonal changes in the pregnancy rates observed on Izu-Oshima were similar to those in Kamakura, Kanagawa Prefecture (Tamura 1989; estimated from the dates litters were weaned). In Taiwan, however, pregnancies peaked during the period from January to March, and also from May to August, two months earlier than on Izu-Oshima and in Kamakura (T'sui et al. 1982).

Although the number of fetuses recorded ranged from one to four with a mean of

Table 2. The number of fetuses observed among 44 pregnant females.

	No. fetuses				
	1	2	3	4	Total
No. females	4	23	15	2	44

2.4±0.7 (*SD*) (see Table 2), the number of weaned juveniles observed in Kamakura, Japan, was one or two with a mean of 1.3±0.5 (*SD*; $n=47$), and in Kenting, Taiwan, the number of weaned juveniles was 1.1±0.3 (*SD*; $n=29$) (Tamura 1989).   In neither Kamakura nor Kenting did I observe females accompanied by more than two weaned juveniles, indicating that not all neonates survive to be weaned.

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